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The purpose of this contract is to study the effects of healthy human pregnancy on cardiac autonomic function (Study #1), oxygen uptake kinetics (Study #2), and acid-base regulation (Study #3) at rest and during upright cycling at intensities above and below the ventilatory anaerobic threshold (T_{VENT}). Study #1 is currently in progress and results to date support our hypotheses that cardiac parasympathetic nervous system activity is blunted in the resting state and that sympathetic activity is reduced during strenuous exercise above T_{VENT} in late gestation. Technical aspects of the study and the testing protocol have been finalized for Study #2 and data collection will begin in January 1998. Study #3 is essentially complete and supports the concept that arterialized plasma $[H^+]$ is lower in the pregnant *vs.* nonpregnant state. However, changes in $[H^+]$ induced by standardized strenuous exercise are comparable in the pregnant *vs.* nonpregnant state. Nonpregnant subjects may have greater reductions in the strong ion difference ($[SID]$) in response to exercise above T_{VENT}, but this is compensated by a greater respiratory response and a reduced arterial plasma carbon dioxide tension. Our results to date support the hypothesis that healthy physically active women can safely adapt to short bouts of strenuous exercise in late gestation.

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TABLE OF CONTENTS

I. INTRODUCTION

Traditional medical advice has been for pregnant women to rest throughout gestation. However, during the past decade there has been increasing participation of women in sports and fitness activities (Mottola and Wolfe, 1994), as well as employment of women in nontraditional occupations (e.g., military services, police work, fire fighting) that involve strenuous physical activities (Fox *et al.*, 1977; Ramirez *et al.*, 1990). When such women become pregnant, it is important to know the modalities, intensities, and durations of physical activity that help to promote maternal and fetal health. It is also important to know the effects of pregnancy on maternal exercise capacities to maintain a safe and productive working environment.

With specific reference to military service, a firm scientific basis is needed to formulate specific policies for the duration of pregnancy leave, assignment of tasks involving strenuous exertion, and formulation of guidelines to maintain physical fitness during the childbearing year. This is particularly important, since active duty military pregnancy has been identified as a high risk category. Risks associated with this population have included a much higher than average risk of preterm labour, toxemia/pre-eclampsia (Fox *et al.*, 1990).

Research over the past decade has established that regular moderate physical activity is both safe and beneficial to women experiencing normal pregnancies. However, basic information is still needed on responses of active pregnant women and their fetuses to strenuous physical activity. The present proposal focuses on cardiovascular control, respiratory gas exchange kinetics, acid-base regulation, and specific hormonal responses to strenuous exercise above the ventilatory anaerobic threshold. Fetal responses to maximal exercise testing in late gestation will also be studied.

The present report is organized to provide separate progress reports (written in journal article format) for each of the three studies that this contract entails:

Study #1   Cardiac Autonomic Function

Study #2   Respiratory Gas Exchange Kinetics

Study #3   Acid-Base Physiology

In accordance with our original Statement of Work (Appendix A), Study #3 is essentially complete and detailed results are presented and discussed. Planning for Study #2 has been completed but data collection has not been initiated. Therefore, our report is limited to a detailed description of methodology. Study #3 is in progress and results to date are presented in detail along with a brief description of our preliminary findings. The final report for this contract will use the same format, but will provide detailed findings and discussion for all three studies.

II. BODY OF REPORT

STUDY #1
CARDIAC AUTONOMIC FUNCTION

BACKGROUND/PURPOSE

As described in a recent scientific review by the principal investigators (Wolfe et al., 1994a), strong support exists for the existence of dose-response relationships between the quantity and quality of maternal aerobic exercise and both maternal and fetal well-being.

Appropriate intensities, durations and frequencies of exercise have been shown to preserve or increase maternal metabolic and cardiopulmonary capacities (Wolfe and Mottola, 1993). Other benefits of such exercise may include facilitation of labour (especially in primiparas), promotion of appropriate maternal weight gain, prevention of gestational diabetes mellitus, and various psychological benefits (Wolfe et al., 1994a). Some recent evidence also suggests that beneficial fetal and placental adaptations may also occur (Clapp and Rizk, 1992; Webb et al., 1994). Conversely, it is also clear that chronic maternal overexertion can cause competition between contracting maternal skeletal muscle for blood flow, oxygen delivery and the availability of essential metabolic substrates (especially glucose). Under such circumstances, maternal physiological needs usually receive a higher priority and intrauterine growth retardation and/or altered fetal development may result (Wolfe et al., 1994a). Thus, the availability of accurate and reliable methods for the prescription and monitoring of maternal exercise intensity is very important to ensure the safety of occupational work and recreational physical activity.

The area of greatest controversy for maternal aerobic exercise prescription has been the use of maternal heart rate to monitor exercise intensity (Caldwell and Jopke, 1985; Gauthier, 1986; White, 1992; Wolfe and Mottola, 1993). A safe upper limit of 140 beats/min has been recommended by the American College of Obstetricians and Gynecologists (1985). However, no research findings have ever been identified to confirm the validity of this recommendation. Practical experience also suggests that aerobic exercise below 140 beats/min is often too low to maintain aerobic fitness in women who are accustomed to regular physical activity prior to pregnancy. Finally, descriptive findings from recent research studies (cited below) also clearly demonstrate the heart rate responses to both acute exercise and physical conditioning are different during pregnancy vs. the nonpregnant state. Thus, more detailed studies are urgently needed to clarify mechanisms of cardiovascular control during pregnancy and to provide an understanding of the true relationship between maternal heart rate and exercise intensity.

8

At rest, heart rate is significantly higher than in the nonpregnant state. This effect begins in the first trimester and gradually increases to approximately 15 beats/min above nonpregnant values by the third trimester (Clapp, 1985; Clapp et al., 1988; Capeless and Clapp, 1989). The degree of heart rate elevation due to pregnancy also appears to diminish with increasing exercise intensity (Wolfe et al., 1990; Guzman and Caplan, 1970). Maximal exercise heart rate is also reported to be reduced (Wiswell et al., 1985; Lotgering et al., 1992). The net effect of these changes is a blunted heart rate response to a given exercise stimulus and reduced maximal heart rate reserve.

The mechanism of heart rate changes during pregnancy has not been thoroughly investigated. Clapp (1985) speculated that the initial increase in resting heart rate in early pregnancy is an effect of hCG and that subsequent increases are reflex responses to other hemodynamic effects of pregnancy (eg., augmented venous capacitance and blood volume, reduced peripheral vascular resistance). Blunted heart rate responses to exercise suggest that baroreceptor responsiveness may be reduced, but studies by Leduc et al. (1991) involving pharmacological manipulations suggest that baroreflex sensitivity is increased. The data of Bonen et al. (1992) further suggest that sympathoadrenal responses to strenuous exercise are also blunted. It seems clear that systematic studies of autonomic balance during graded exercise would be a logical first step to explain heart rate responses to exercise in pregnancy.

Changes in heart rate at rest and during exercise depend on the interaction between parasympathetic/vagal and sympathetic autonomic influences on the SA node. Pharmacological and physiological studies support the hypothesis that electrocardiographic R-R intervals vary on a beat-to-beat basis, depending on the degree of both sympathetic and parasympathetic influences (Yamamoto et al., 1991; Yamamoto and Hughson, 1991). Studies which have employed spectral analysis of heart rate variability (HRV) further support the concept that high frequency HRV (> 0.15 Hz) is the result of cardiac parasympathetic influences whereas low frequency HRV (< 0.15 Hz) results from both sympathetic and parasympathetic autonomic activity (Akselrod et al., 1981, 1985; Berger et al., 1989; Pagani et al., 1986). Also, the ratio of low:high HRV may be a valid indicator of sympathetic activity by itself (Pagani et al., 1986; Pomeranz et al., 1985).

Spectral analysis of HRV has recently been used to quantify cardiac autonomic activity during pregnancy. The findings of Ekholm et al. (1992) demonstrated a decrease in HRV in the high frequency peak at rest, suggesting a decreased vagal tone compared to nonpregnancy controls. Their observations, along with more recent reports (Ekholm et al., 1993) of an overall decrease in HRV measured both at rest and during controlled breathing, support the hypothesis that parasympathetic nervous system (PNS) activity is decreased during pregnancy in the resting state. However, in response to an orthostatic test, pregnant subjects demonstrated an increase in PNS activity compared to the expected sympathetic activation found in control subjects (Ekholm et al., 1992). The authors hypothesized that the higher sympathetic nervous system activity and lower PNS activity observed at rest during pregnancy results in an attenuated sympathetic response to standing up. To date, HRV spectral analysis has not been used to examine cardiac autonomic balance during exercise in pregnancy in any published study.

Eneroth-Grimfors *et al.* (1994) employed HRV spectral analysis techniques to investigate the importance of altered cardiac autonomic function as an important etiologic factor in pre-eclampsia. Their data suggest that vagal activity is reduced in pre-eclamptic subjects in the resting state compared with healthy pregnant and nonpregnant controls. To date, there have been no published studies on the effects of exercise on cardiac autonomic activity in healthy pregnant women or in women with pre-eclampsia. However, Jones *et al.* (1992) have demonstrated the efficacy of regular exercise to preserve reproductive tissue blood flow in hypertensive pregnant rats.

In summary, both pregnancy and acute exercise alter cardiac autonomic activity. Therefore, the measurement of HRV in combination with analysis of plasma catecholamines (epinephrine, norepinephrine) will greatly enhance the amount of information that can be gained from exercise tests conducted during late gestation. The present study will provide normative data on cardiac autonomic responses to exercise in pregnancy and will facilitate future grant applications for the investigation of causes and treatments for pre-eclampsia.

EXPERIMENTAL DESIGN

Subjects will be 30 nonsmoking, healthy, physically active pregnant women (gestational age, 34-38 weeks). Nonpregnant control data are being obtained by retesting the subjects following complete recovery from childbirth in the nonlactating state (approximately 6 months postpartum).

A reference group of 30 healthy nonsmoking women with similar mean ages, parity, body heights, and physical activity levels is also being studied. Test results from the reference group will be compared to those of postpartum evaluation to verify the validity of postpartum measurements for use as nonpregnant control data.

Recruitment methods for the ***Experimental (Pregnant) Group*** have included newspaper ads and posted announcements, as well as contact with local obstetricians and community agencies which provide services to women. Each prospective subject is being screened medically by the obstetrician monitoring her pregnancy and has provided written informed consent. A standard medical screening form (Addendum A) developed by our research group for the Ontario Fitness Safety Standards Committee (1990) will be employed. Information on this form is provided by the subject herself and the physician monitoring her pregnancy (at her specific request). Specific criteria for inclusion in the ***Experimental (Pregnant) Group*** are described in Table 1-A, 1-B.

Members of the ***Control Group*** are healthy young nonpregnant women. Subjects have been recruited from the same general population as pregnant subjects via newspaper ads and posted announcements. Specific inclusion criteria will be the following: Age, 20-40 years; physically active; parity - 0 to 2; nonsmoker; nonobese/nondiabetic; taking no medications; good general health (as determined by Physical Activity Readiness Questionnaire).

TABLE 1-A INCLUSION CRITERIA	TABLE 1-B CONTRAINDICATIONS TO EXERCISE TESTING AND CONDITIONING IN PREGNANCY†	
	Absolute	**Relative**
Age, 20 - 40 years Parity 0-2 Physically Active Nonsmoker Taking no medications other than prenatal vitamins Absence of Contraindications to Exercise†	♦ Clinically significant valvular or ischemic heart disease. ♦ Type I diabetes mellitus, peripheral vascular disease, thyroid disease or uncontrolled hypertension, other serious system disorder. ♦ An incompetent cervix (multigravid patients). ♦ A history of two or more spontaneous abortions. ♦ Bleeding or placenta previa. ♦ Ruptured membranes, premature labour. ♦ Toxemia or pre-eclampsia (current pregnancy). ♦ Evidence of fetal growth retardation (current pregnancy). ♦ Very low % of body fatness, eating disorder (anorexia, bulimia). ♦ A multiple pregnancy (eg., triplets).	♦ History in previous pregnancies of premature labour, intrauterine growth retardation. ♦ Anemia or iron deficiency (Hb < 10 g/dl) ♦ Clinically significant pulmonary disease (eg., COPD). ♦ Mild valvular or ischemic heart disease, significant cardiac arrhythmia. ♦ Very low physical fitness prior to pregnancy. ♦ A prescription of drugs which can alter cardiac output or blood flow distribution. ♦ Obesity and/or Type II diabetes prior to pregnancy.

† References:
Ontario Fitness Safety Standards Committee (1990);
American College of Sports Medicine (1991);
Mottola and Wolfe (1993).

TECHNICAL OBJECTIVES/HYPOTHESES

♦ To study the effects of pregnancy on HRV at rest and during steady-state cycle ergometer exercise. **_Hypothesis:_** Changes in HRV characteristics will reflect reduced vagal/parasympathetic cardiac influences in the resting state and blunted sympathetic influences during exercise. Significant correlations will be observed between HRV low:high frequency power and plasma catecholamine responses (norepinephrine, epinephrine) during exercise above the ventilatory anaerobic threshold (T_{VENT}).

♦ To study the effects of pregnancy on spontaneous baroreflex (SBR) sensitivity in the resting state. **_Hypothesis:_** SBR sensitivity will be reduced at rest, suggesting reduced resting vagal/parasympathetic influence in the pregnant *vs.* nonpregnant state.

METHODOLOGY

Participation for each subject involved 2 visits to the Clinical Exercise Physiology Laboratory at Queen's University. The initial visit was used to explain medical screening and testing procedures and to familiarize subjects with the laboratory. Basic anthropometric measurements, including body height, body mass, and the maximal exercise test described below were also performed on the first visit. Caloric expenditure during occupational and recreational physical activity was quantified using a validated questionnaire (Bouchard *et al.*1983; Paffenbarger *et al.*, 1993). The final visit consisted of one 2.5 h testing session conducted at least 3 days after the first visit.

Subjects abstained from caffeine and alcohol intake 6 hours prior to testing and avoided strenuous exercise for at least 12 h prior to testing. They consumed a standard meal (350 kcal, 40% protein, 40% carbohydrate, 20% fat) 1 hour before the tests. Room temperature and relative humidity were controlled and recorded at the time of each test. A fan was placed in front of the subject during exercise testing and both lighting and number of testers was kept constant for each subject. Finally, the noise level was minimized during the resting tests, with interruptions being documented.

Exercise Testing

Basic physical measurements included resting blood pressure, body height, body mass and forced vital capacity (Cavitron SC-20A spirometer).

Exercise tests were performed in the upright posture on a Sensor Medics (Model 800) constant work rate cycle ergometer (60 rpm). For the nonpregnant control group, the first exercise test was used to determine maximal aerobic power ($\dot{V}O_2$ max) using a progressive protocol that involved a 4 minute warm up at 20 watts followed by 20 watts/min ramp increase in work rate until fatigue (Lotgering *et al.*, 1992; Kemp *et al.*, 1997). T_{VENT} for all subjects was determined using the V-slope method (Beaver *et al.*, 1986). Oxygen pulse at a heart rate of 170 beats/min was used as an index of aerobic working capacity (Wolfe *et al.*, 1994b).

Subjects performed a 2-stage submaximal exercise test on a separate day. HRV was evaluated at rest in both the left lateral decubitus and sitting postures at a breathing frequency of 16 breaths/min (subjects were asked to breathe in time to a buzzer hooked up to a breath rate timer). The test in the left lateral decubitus posture preceded the seated posture test in order to elicit an increase in sympathetic activation for evaluation of the effect of pregnancy on this aspect of HRV. Subjects rested in the left lateral position for at least 2 minutes before data collection began. After resting data collection, the exercise testing protocol involved a 3 minute warm up at 20 watts, followed by a 20 watts/min ramp increase in work rate to a level corresponding to 60% of the work rate at T_{VENT}. After approximately 20 minutes rest and 3 minute warmup, the subjects performed a second work bout at 110% of T_{VENT}.

During the 2-stage submaximal test, exercise was performed for 12 and 10 minutes (including the warm-up) at 60% and 110% of the work rate at T_{VENT}, respectively to obtain sufficient data (≥ 512 heart cycles) for HRV and SBR analyses (Yamamoto *et al.*, 1991). Blood pressure in the finger (Ohmeda 2300 Finapres) was measured continuously during the protocol. During data collection the

servo reset mechanism of the Finapres was turned off so that continuous blood pressure could be recorded (Iellamo *et al.*, 1994; Hughson *et al.*, 1995). Also, both pulse rate and pressure alarms were turned off to avoid sudden disturbances which might have influenced test results. Respiratory responses (f, V_T) and $\dot{V}O_2$ were measured breath-by-breath during both submaximal exercise bouts as well as the progressive cycle exercise test using a computerized system (First Breath Inc.) that incorporates a respiratory mass spectrometer (Perkin-Elmer, MGA-1100) with a volume turbine (Alpha Technologies VMM-110) (Hughson *et al.*, 1991).

Heart Rate Variability Spectral Analysis

Briefly, for each of the resting tests, and both submaximal exercise tests the TTL R-wave output from a Marquette Max-1 exercise electrocardiograph (ECG) was recorded continuously and stored using a computerized system. HRV was evaluated at rest and during constant work rate exercise using coarse-graining spectral analysis of HRV to generate both time domain analysis (calculation of mean R-R ± SD), and frequency domain analysis. After HRV data files had been edited, they underwent fast-Fourier transformation to plot the R-R interval spectrum. HRV variables, including PNS and SNS indicators, total power, and fractal power were then recorded. As described above, pulmonary ventilation and breathing frequency were also measured by open circuit spirometry to monitor changes in respiratory responses that may alter HRV spectral parameters (Brown *et al.*, 1993).

Spontaneous Baroreflex Function

The analysis of SBR function involved the simultaneous collection of heart rate (Max-1 ECG) and systolic blood pressure (SBP) (Ohmeda 2300 Finapres) data on a beat-to-beat basis during both postures at rest, and during both submaximal exercise loads. Indices of SBR function, including mean slope, mean R-R interval, mean SBP, and total number of segments were calculated and recorded for each test (Parati *et al.*, 1988; McDonald *et al.*, 1993). The test-retest reliability of both HRV and SBR variables measured at rest and during exercise in healthy nonpregnant subjects using the present methods has been confined in a recent publication from this laboratory (Amara and Wolfe, in review).

Blood Biochemistry

Venous blood samples were drawn from an indwelling catheter in the antecubital vein by a registered nurse for the determination of plasma lactate concentration. During the progressive cycle exercise test, blood was drawn (approximately 5 ml) at rest, and at 1, 3, 5, 7, 10, and 15 minutes post-exercise. During the 2-stage submaximal protocol, samples were also obtained during the last minute at rest (sitting posture) and during the last minute of exercise at both 60% and 110% T_{VENT}. Samples were treated with an anticoagulant (potassium oxalate) and an antiglycolytic agent (sodium fluoride), centrifuged (IEC Centra-MP4R) at 4 degrees Celsius, and frozen for later analyses using an automated analyzer (Yellow Springs Instruments, Model 23L).

Venous blood samples for the determination of plasma catecholamine content will be obtained using an indwelling catheter inserted 30 minutes prior to the start of each of the 2 submaximal exercise tests. Samples will be obtained in the resting state and at the end of each steady-state exercise bout. Samples were transferred into a 5 ml vacutainer containing EDTA and 5 mg glutathione, mixed,

centrifuged and plasma stored (-86 degrees C) until assayed. Epinephrine and norepinephrine content will be determined by high performance liquid chromatography as described by Foti *et al.* (1987).

Statistical Analyses

Physical and demographic characteristics (age, body height, body mass prior to pregnancy, parity, physical activity levels prior to pregnancy) and simple exercise variables of the pregnant and nonpregnant groups will be compared using conventional parametric (unpaired Student *t*-statistic) or nonparametric (Chi square analysis) tests.

Responses to exercise will be analyzed using a two-way analysis of variance for repeated measures (groups x work rate). Student-Newman-Keuls multiple range tests will be used to compare main effect means when significant F-ratios are obtained. Regression analysis will also be used to describe statistical relationships between experimental variables within and between groups (eg., comparison of heart rate variability low:high frequency area *vs.* plasma catecholamines in pregnant *vs.* nonpregnant subjects).

<u>Note:</u> **Results obtained to date (means ± SE) are provided below. Statistical analyses described above will be performed at the conclusion of data collection for this study.**

RESULTS

Subjects

To date, 7 members of the pregnant group (n = 7) have successfully completed the experimental protocol. Data from one member of the control group (n = 10) was not included in the results for the left lateral decubitus posture due to technical problems with data acquisition. In addition, blood samples were drawn from only 6 out of the 7 pregnant participants because one subject asked to be excluded from this part of the testing procedure.

Mean values for age, prepregnant body mass, body height, and prepregnant body mass index (BMI) were moderately higher in the pregnant group (Table 2). Values for O_2 pulse at 170 bets/min and T_{VENT} were similar. As expected, body mass and BMI were higher also for the pregnant group than for the control group. Of the 10 nonpregnant control subjects tested to date, 4 were in the luteal phase of the menstrual cycle at the time of 2-stage submaximal exercise test and the remaining 6 were in the luteal phase.

Table 2 **Physical Characteristics of Subjects**

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Age (years)	30.7 ± 1.1	26.9 ± 1.6
Body Height (cm)	166.9 ± 1.9	162.1 ± 1.9
Body Mass (kg)	76.4 ± 3.6	58.9 ± 2.1
Prepregnancy Body Mass (kg)	69.6 ± 2.6	N/A
Body Mass Index	27.5 ± 1.5	23.2 ± 1.0
Prepregnancy Body Mass Index	25.1 ± 1.2	N/A
Gestational Age (weeks)	32.9 ± 1.9	N/A
O_2 pulse at 170 beats/min (ml/beat)	14.6 ± 1.5	13.4 ± 0.4
T_{VENT} (L/min)	1.71 ± 0.04	1.79 ± 0.07

Values are means ± SE.
N/A = not applicable.

HRV Spectral Analyses

Mean values (± SE) for HRV spectral parameters at rest in the seated posture and left lateral decubitus posture appear in Tables 3 and 4, respectively. Similar data trends were observed for both postures. Results suggest similar values for low frequency power and percent fractal power between the pregnant and nonpregnant control groups. Pregnant values for high frequency power, total harmonic power, fractal power, total power, and the PNS indicator were considerably lower (89%, 82%, 83%, and 43%, respectively) than corresponding control values, whereas the SNS indictor was much higher (4.83 ± 2.19 *vs.* 0.72 ± 0.17 ms^2/Hz) in pregnant subjects *vs.* control subjects during the seated posture.

The effects of submaximal cycle exercise at 60% T_{VENT} on HRV spectral parameters appear in Table 5. The pregnant group showed higher total power (129 ± 50 *vs.* 94 ± 21 ms^2/Hz) than the control group, but all other variables were similar between groups at this exercise intensity.

Mean values for HRV spectral variables at 110% T_{VENT} are shown in Table 6. Only pregnant values are shown because of a technical problem related to quantification of high frequency power and calculation of the SNS indicator in members of the nonpregnant control group. In this regard, high frequency power values were very low in control group subjects, suggesting complete or nearly complete withdrawal of parasympathetic/vagal tone. Due to a software limitation, this resulted in expression of high frequency power as zero (as opposed to a very small number). Since the SNS indicator is calculated as low frequency power divided by high frequency the SNS indicator was *"infinity"* - suggesting a very high number for each subject that exceeded the mean value for the SNS indicator in the pregnant group. As expected, SNS indicator values at 110% T_{VENT} (15.21 ± 9.29 m²/Hz) than during exercise at 60% T_{VENT} (10.63 ± 3.73 ms²/Hz).

Table 3   **HRV Spectral Parameters in the Seated Posture**

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Low Frequency Power (ms²/Hz)	166 ± 50	401 ± 107
High Frequency Power (ms²/Hz)	157 ± 105	1362 ± 653
Total Harmonic Power (ms²/Hz)	324 ± 101	1764 ± 705
Fractal Power (ms²/Hz)	497 ± 141	3188 ± 1102
Fractal Power (%)	60 ± 8	64 ± 4
Total Power (ms²/Hz)	821 ± 208	4952 ± 1661
PNS Indicator (High/Total)	0.13 ± 0.06	0.23 ± 0.04
SNS Indicator (Low/High)	4.83 ± 2.19	0.72 ± 0.17

Values are means ± SE.

Table 4   **HRV Spectral Parameters in the Left Lateral Decubitus Posture**

Variable	Pregnant Group (n = 7)	Control Group (n = 9)
Low Frequency Power (ms^2/Hz)	123 ± 79	635 ± 282
High Frequency Power (ms^2/Hz)	160 ± 81	3971 ± 2476
Total Harmonic Power (ms^2/Hz)	1856 ± 1728	4618 ± 2750
Fractal Power (ms^2/Hz)	588 ± 153	2511 ± 701
Fractal Power (%)	70 ± 9	50 ± 5
Total Power (ms^2/Hz)	843 ± 214	7130 ± 3314
PNS Indicator (High/Total)	0.16 ± 0.05	0.37 ± 0.06
SNS Indicator (Low/High)	1.37 ± 0.57	0.48 ± 0.19

Values are means ± SE.

Table 5   **HRV Spectral Parameters During Cycle Exercise at 60% T$_{VENT}$**

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Low Frequency Power (ms^2/Hz)	39 ± 19	31 ± 15
High Frequency Power (ms^2/Hz)	7 ± 3	7 ± 3
Total Harmonic Power (ms^2/Hz)	47 ± 22	42 ± 17
Fractal Power (ms^2/Hz)	82 ± 39	52 ± 6
Fractal Power (%)	66 ± 8	67 ± 7
Total Power (ms^2/Hz)	129 ± 50	94 ± 21
PNS Indicator (High/Total)	0.04 ± 0.01	0.07 ± 0.02
SNS Indicator (Low/High)	10.63 ± 3.73	12.21 ± 7.00

Values are means ± SE.

Table 6   HRV Spectral Parameters During Cycle Exercise at 110% T_{VENT}

Variable	Pregnant Group (n = 7)	* Control Group (n = 10)
Low Frequency Power (ms^2/Hz)	0.4 ± 0.2	
High Frequency Power (ms^2/Hz)	0.09 ± 0.04	
Total Harmonic Power (ms^2/Hz)	3.3 ± 0.4	
Fractal Power (ms^2/Hz)	10 ± 3	
Fractal Power (%)	71 ± 5	
Total Power (ms^2/Hz)	13 ± 3	
PNS Indicator (High/Total)	0.006 ± 0.002	
SNS Indicator (Low/High)	15.21 ± 9.29	

Values are means ± SE.

* See comments on page 16.

Respiratory Responses to Exercise

Respiratory data recorded during the two bouts of submaximal exercise are represented in Table 7. Mean values for breathing frequency were similar between the pregnant group and the control group during exercise at 60% and 110% of T_{VENT}. As expected, tidal volumes were higher in pregnancy at both exercise intensities compared with control data.

Table 7   **Respiratory Responses to Submaximal Steady-State Exercise**

Condition	Variable	Pregnant Group	Control Group
60% T_{VENT}	f	27.2 ± 3.2	25.2 ± 1.2
	V_T	1349 ± 71	1164 ± 81
110% T_{VENT}	f	33.8 ± 3.7	30.9 ± 2.0
	V_T	2051 ± 81.1	1812 ± 92

f-breathing frequency (breaths/min); V_T - tidal volume (ml)
Values are means ± SE.

Spontaneous Baroreflex Function

SBR function was analyzed at rest during the seated posture (Table 8) and while lying in the left lateral decubitus posture (Table 9), by examining values for total number of segments, mean slope (ms/mmHg), mean R-R interval (ms), and mean SBP (mmHg). Similar trends were found for both resting postures. The total number of segments was similar for the two groups. Mean slope was considerably lower for both sitting and left lateral decubitus resting measures in the pregnant group (8.6 ± 1.8 and 10.5 ± 1.7, respectively) compared with control data (19.7 ± 4.7 and 29.1 ± 4.9, respectively). Both mean R-R interval and mean SBP were slightly lower in the pregnant group compared with nonpregnant control group.

SBR data from cycle exercise at 60% T_{VENT} are found in Table 10. All SBR variables at this exercise intensity were similar in both pregnant and control groups. Indices of SBR function (Table 11) during exercise at 110% T_{VENT} were similar for both control and pregnant groups. The slopes were slightly lower, and R-R interval shorter for both groups during exercise at 110% T_{VENT} compared to exercise at 60% T_{VENT}.

Table 8   SBR Function at Rest in the Seated Posture

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Total Number of Segments	100 ± 17	118 ± 20
Mean Slope (ms/mmHg)	8.6 ± 1.8	19.7 ± 4.7
Mean R-R (ms)	726 ± 36	979 ± 58
Mean SBP (mmHg)	113 ± 5	127 ± 4

Values are means ± SE.

Table 9   SBR Function at Rest in the Left Lateral Decubitus Posture

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Total Number of Segments	73 ± 11	72 ± 15
Mean Slope (ms/mmHg)	10.5 ± 1.7	29.1 ± 4.9
Mean R-R (ms)	769 ± 33	1074 ± 71
Mean SBP (mmHg)	100 ± 8	105 ± 6

Values are means ± SE.

Table 10   SBR Function During Cycle Exercise at 60% T_{VENT}

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Total Number of Segments	49 ± 15	55 ± 5
Mean Slope (ms/mmHg)	1.8 ± 0.3	1.5 ± 0.2
Mean R-R (ms)	549 ± 35	555 ± 32
Mean SBP (mmHg)	147 ± 11	140 ± 9

Values are means ± SE.

Table 11   SBR Function During Cycle Exercise at 110% T_{VENT}

Variable	Pregnant Group (n = 7)	Control Group (n = 10)
Total Number of Segments	41 ± 4	34 ± 6
Mean Slope (ms/mmHg)	1.0 ± 0.1	1.1 ± 0.1
Mean R-R (ms)	376 ± 6	391 ± 18
Mean SBP (mmHg)	160 ± 12	172 ± 12

Values are means ± SE.

Lactate concentration at rest and during exercise did not differ between the pregnant group and the control group (Table 12). Blood lactate concentration was lowest at rest and rose with increasing exercise intensity. As expected values obtained at 60% T_{VENT} were below 4.0 mmol/L, and those at 110% T_{VENT} were above 4.0 mmol/L. This confirms that the two work rates were above and below the onset of blood lactate accumulation (OBLA), respectively in both groups.

Table 12   Venous Blood Lactate Values at Rest and During Exercise

Condition	Pregnant Group (n = 4)	Control Group (n = 5)
Rest	0.96 ± 0.13	1.30 ± 0.27
60% T_{VENT}	2.04 ± 0.35	1.55 ± 0.30
110% T_{VENT}	6.48 ± 0.51	6.98 ± 1.32

Values are means (mmol/L) ± SE.

Note: *Plasma lactate analyses for 3 pregnant subjects and 5 control subjects are currently in progress.*

Blood samples have been collected at rest and at the end of the exercise bouts at 60% and 110% of T_{VENT}. Samples obtained to date are currently in storage in our -86 °C deep freezer. Recent subject recruitment for this study has been strong (a group of 10 new subjects are currently being tested), and the first major batch of samples will be sent to Dr. M.F. Mottola in late November at the University of Western Ontario for analysis using high performance liquid chromatography as described above.

BRIEF DISCUSSION OF RESULTS TO DATE

Our findings to date are consistent with our hypothesis that cardiac parasympathetic/vagal activity is reduced in the resting state in human pregnancy. This is supported by values for high frequency power, the PNS indicator and SBR slope in both the seated and left lateral decubitus postures. Although formal statistical analyses have not be conducted, the mean differences and standard errors of the mean suggest that these between group effects are all statistically significant. The present data also suggest that both HRV and SBR responses to exercise at 60% T_{VENT} are similar in the pregnant *vs.* nonpregnant state.

Further work will be needed to clarify cardiac autonomic responses to exercise at 110% T_{VENT}. Data within the control group show progressive withdrawal of parasympathetic/vagal tone (as reflected by high frequency power, total power, the PNS indicator and SBR slope) in the transition from rest to 60% T_{VENT} and from 60% to 110% T_{VENT} and progressive increases in the SNS indicator during these transitions (keeping in mind that parasympathetic/vagal responses were blunted at rest).

As described above, values for high frequency power were very low at 110% T_{VENT} for all members of the nonpregnant control group, resulting in values of zero on our computer printout. Use of these values to calculate the SNS indicator (low/high frequency power) results in very high values (i.e., infinity). This suggests, as we hypothesized, that values for sympathetic activity were higher than in the pregnant group. Manual analysis of HRV spectra in the nonpregnant control group will help to circumvent this problem. Plasma norepinephrine data will also be helpful to answer this question.

BACKGROUND/PURPOSE

Gas Exchange Kinetics During Nonsteady-State Exercise

Human pregnancy involves a number of adaptations that could, in theory, alter the integrated cardiorespiratory response to changes in metabolic demand. These include substantial increases in blood volume, altered arterial and venous blood gas tensions ($\uparrow$ PO_2, $\downarrow$ PCO_2), increased respiratory sensitivity to CO_2, and hyperkinetic hemodynamics both at rest and during exercise (Wolfe *et al.*, 1989; Wolfe and Mottola, 1993).

Methods for the study of respiratory gas exchange kinetics have been significantly enhanced over the last 10-15 years by the development of computerized breath-by-breath metabolic measurement systems. Current practice requires the performance of several repetitions of the exercise protocol for each subject with continuous breath-by-breath sampling (Whipp *et al.*, 1982; Hughson *et al.*, 1988; Murphy *et al.*, 1989). To minimize *"biological noise,"* the data are linearly interpolated and ensemble-averaged to provide a single data set. Sophisticated mathematical models are then employed to calculate variables such as the time lag, gain and time constant for the response to a change in work rate (Whipp *et al.*, 1982; Hughson *et al.*, 1988; Murphy *et al.*, 1989).

To date, only one study has examined respiratory gas exchange during nonsteady-state exercise in pregnancy using breath-by-breath technology. Edwards *et al.* (1991) measured changes in minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$), and carbon dioxide output ($\dot{V}CO_2$) during the transition from the resting state to achievement of a steady-state during 6 minutes of upright cycling at a power output of 50 watts. Twenty healthy women were studied at 38 weeks gestation and 3 months postpartum. The protocol was administered only once at each testing time. During pregnancy, the subjects exhibited greater changes in $\dot{V}_E$ and more rapid changes in $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ in the transition from rest to steady-state exercise. Although a valid mathematical model of $\dot{V}O_2$ kinetics was not employed, their results suggested that pregnant women exhibit greater respiratory gain (as reflected by higher values for $\Delta \dot{V}_E / \Delta CO_2$), and a faster time constant for $\dot{V}O_2$ kinetics.

The *"slow"* (or Phase III) component of oxygen uptake kinetics has received a considerable amount of attention in recent scientific publications from the field of exercise physiology (Barstow *et al.*, 1994; Whipp *et al.*, 1994; Gaesser and Poole, 1996). Briefly, following the initial adjustments (Phase I, Phase II) to sustained heavy exercise above the ventilatory anaerobic threshold (T_{VENT}) increases in $\dot{V}O_2$, $\dot{V}_E$ and heart rate (HR) continue in a slow and progressive fashion until fatigue. The changes in $\dot{V}O_2$ are out of proportion to the apparent metabolic demand of a work rate being performed. This effect has been attributed to the recruitment of low efficiency fast twitch muscle fibers (Gaesser and Poole, 1996) and changes in $\dot{V}O_2$ are reported to correlate significantly with increase in blood lactate concentration (Kowalchuk *et al.*, 1997). To date, no investigations have examined the slow component of oxygen uptake kinetics. However, since blood lactate responses to heavy exercise

appear to be blunted in late gestation (Clapp *et al.*, 1987; McMurray *et al.*, 1988; Wolfe *et al.*, 1994b), we hypothesize that oxygen uptake kinetic responses to heavy exercise are slower in the pregnant *vs.* nonpregnant state.

TECHNICAL OBJECTIVES/HYPOTHESES

♦ To study the effects of human pregnancy on Phase II oxygen uptake kinetics in response to a step increase in work rate from loadless pedaling to a work rate corresponding to 80% of the ventilatory anaerobic threshold (T_{VENT}). ***Hypothesis:*** The amplitude of increase (a) for $\dot{V}O_2$ will be unaffected by pregnancy but the time delay (δ) will be significantly shorter and the time constant (τ) will be significantly faster in the pregnant *vs.* the nonpregnant state.

♦ To study the effects of human pregnancy on the slow component of oxygen uptake kinetics following a step increase in work rate to a work rate corresponding to 120% of T_{VENT}. ***Hypothesis:*** The $\dot{V}O_2$ kinetic response to exercise at 120% of T_{VENT} will be slower in the pregnant *vs.* nonpregnant state. This response will be associated with lower plasma lactate levels and a slower rate of blood lactate accumulation during pregnancy.

METHODOLOGY

The study design involves the testing of 12 nonsmoking, healthy, physically active pregnant women (gestation ages 34-38 weeks) and 12 nonpregnant control subjects. The two groups will be matched for important physical characteristics including age, parity, body height, prepregnant body mass, skin fold thicknesses (Taggart *et al.*, 1967) and maximal oxygen uptake. Control subjects will be women with normal menstrual cycles who are not taking oral contraceptives. Menstrual cycle phase during the exercise tests described below will be accurately determined for each control subject.

Both pregnant and nonpregnant control subjects will perform a series of 5 exercise tests. The first test will be a progressive maximal cycle ergometer protocol with a ramp increase in work rate of 20 watts/min until volitional fatigue (Lotgering *et al.*, 1992; Kemp *et al.*, 1997). Respiratory responses to exercise will be evaluated on a breath-by-breath basis using a computerized system (Hughson *et al.*, 1991) that incorporates a respiratory mass spectrometer (Perkin-Elmer MGA-1100) with a volume turbine (Alpha Technologies VMM-1100). The algorithm of Beaver *et al.* (1981) will be used to calculate breath-by-breath gas exchange and the V-slope method (Beaver *et al.*, 1986) will be used to identify the ventilatory anaerobic threshold (T_{VENT}).

The remaining 4 cycle ergometer exercise test sessions will use the same protocol and will be performed on separate days (maximum time between tests, 3 days). Each test will involve a period of resting data collection, followed by a step increase in work rate from loadless pedaling (4 min.) to a work rate corresponding to 80% at T_{VENT} for 8 minutes. After a 20 minute rest period, the subject will perform a second exercise test that involves a step increase from loadless pedaling to a work rate corresponding to 120% of T_{VENT} for 8 minutes (or volitional fatigue). In 2 of the 4 test sessions, arterialized blood samples will be obtained at rest and at 2 minute intervals during exercise and the

26

immediate 10 minutes post exercise recovery period for the determination of plasma lactate concentration using an automated analyzer (Yellow Springs Instruments, Model 2300).

Breath-by-breath respiratory gas analysis will be conducted at rest (5 min), during exercise, and during post-exercise recovery (15 min) as described above. Breath-by-breath oxygen uptake data for each of the 4 repetitions at both exercise levels will be linearly interpolated (1.0 s), time aligned and ensemble averaged to provide a single data set for each subject at both exercise levels. Phase II oxygen uptake kinetics at 80% of T_{VENT} will be analyzed in accordance with a monoexponential equation of Whipp et al. (1982):

$$Y(t) = a \{1 - e^{-[(t-\delta)/\tau]}\},$$

where Y represents either $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, or HR above baseline at any time (t); a is the amplitude of increase between the "0" W mean and the response at 3 min of the square wave work rate; τ is the time constant; and δ the delay (35). The slow component (i.e., Phase III) of oxygen uptake kinetics at 120% T_{VENT} will be characterized mathematically and correlated with measures of plasma lactate as described by Barstow (1994) and Kowalchuk et al. (1997).

For maximal cycle ergometer tests involving pregnant subjects, fetal heart rate will be recorded continuously for 20 minutes before exercise, during exercise (where feasible) and for 20 minutes after exercise using Doppler ultrasound (Hewlett-Packard Model 8041-A cardiotocograph). Gross changes in fetal heart rate patterns are employed as clinical indicators of fetal distress. Criteria for evaluation of baseline variability, tachycardia and deceleratory patterns are those described in a recent review (Wolfe et al., 1994). These data will be added to our existing data bank on fetal responses to varying intensities and durations of exercise at different gestational ages (Webb et al., 1994; Wolfe et al., 1994).

Note: *As indicated in our original Statement of Work (Appendix A), we have consulted with expert colleagues at the University of Western Ontario concerning the optimal testing protocol and mathematical model to characterize oxygen uptake kinetics in pregnant women. In accordance with this advice we have decided to employ the monoexponential model of Whipp et al. (1982) to model Phase II kinetics as opposed to the two component model of Hughson et al. (1988). Advice was also received on modelling of the slow/Phase III component of oxygen uptake kinetics in accordance with similar recent work involving elderly subjects (Kowalchuk et al., 1997). Local human ethics review board approval has been received for the study.*

We will be submitting the required documentation for review by the U.S. Army Medical Research and Materiel Command in the immediate future. We expect to begin data collection in January 1998.

STUDY #3
ACID-BASE PHYSIOLOGY

BACKGROUND/PURPOSE

Acid-Base Balance at Rest

Pregnancy is characterized by respiratory alkalosis which has been documented as early as 8 weeks gestation (Anderson et al., 1969; Blechner et al., 1968; Gilbert et al., 1962; Lucius et al., 1970) and is maintained until delivery (Gilbert et al., 1962; Lim et al., 1976; Lucius et al., 1970; Machida et al., 1981; Prowse and Gaensler, 1965). Arterial pH levels between 7.38 and 7.46 at 12-42 weeks gestation have been reported (Blechner et al., 1968), as well as values between 7.44 and 7.52 at 8-42 weeks gestation (Lucius et al., 1970). Lim et al. (1976) reported an average pH of 7.44 in third trimester subjects. At 36 weeks gestation, pH levels have been reported at approximately 7.46 (Moore et al., 1987; Pivarnik et al., 1992), however both studies were conducted at an altitude of 1400-1600 meters where resting ventilation has been shown to increase. The respiratory alkalosis is attributable to pregnancy-induced increases in minute ventilation ($\dot{V}_E$), which lead to a reduction in arterial carbon dioxide tension (P_aCO_2) to approximately 30-32 mm Hg (Anderson et al., 1969; Lim et al., 1976; Lucius et al., 1970; Lyons and Antonio, 1959; Machida et al., 1981; Templeton and Kelman, 1976). These maternal responses to pregnancy, which appear in the first trimester (Anderson et al., 1969; Blechner et al., 1968; Gilbert et al., 1962; Machida, 1981; Rees et al., 1990), may act to facilitate placental gas exchange prior to development of a functional fetal circulatory system (Liberatore et al., 1984).

The respiratory alkalosis described above is partly compensated by renal excretion of bicarbonate (Prowse and Gaensler, 1965; Hytten, 1968), and a lowering of the plasma bicarbonate concentration (Anderson et al., 1969; Blechner, 1993; Dayal et al., 1972; Lucius et al., 1970; Machida, 1981). In accordance with conventional acid-base theory (Cameron, 1989), the decrease in plasma bicarbonate levels, along with relative anemia (Lund and Donovan, 1967) and hypoproteinemia (Pivarnik et al., 1990), leads to a reduced buffering capacity of maternal blood. Consequently, pregnant women may be more prone to metabolic acidosis during strenuous exercise.

Acid-Base Balance During Exercise

The changes in maternal acid-base regulation described above suggest that women may be more prone to metabolic acidosis during pregnancy when working at levels above the onset of blood lactate accumulation (OBLA). This raises further questions concerning the acid-base status of the fetus during strenuous exercise, and the levels of exertion that may be considered safe in late gestation. Limited placental permeability to hydrogen and bicarbonate ions (Blechner et al.m 1967) has been reported and this may assist in protecting the fetus from changes in maternal blood pH. Under normal resting conditions the fetus has a lower pH than maternal blood (Blechner et al., 1967; Blechner, 1993). Alternatively, during times of reduced maternal pH, such as during heavy exercise, the transplacental pH gradient may be eliminated or reversed thereby increasing the likelihood of fetal

28

acidosis.

Only two studies (Lehmann and Regnat, 1976; Pivarnik *et al.*, 1992) have examined changes in maternal plasma pH during exercise in pregnancy using the conventional approach to acid-base analysis. Pivarnik *et al.*(1992) observed that arterial pH values decreased during 6 min of treadmill (67 m/min, 2.5%; 67 m/min, 12% grade), and cycle ergometer work (50 W, 75 W) in both the pregnant and nonpregnant states. The average absolute pH at rest and exercise was greater in pregnancy in each protocol, and the average absolute reduction in pH was also the same in both groups. However, Lehmann and Regnat (1976) reported slightly larger decreases in arterial pH when 6 min of cycling (50 W; 80 W) was performed during pregnancy as well as lower absolute pH values at 80W.

Reported changes in bicarbonate concentration that occur with exercise during pregnancy are controversial. Compared with postpartum, decreases in bicarbonate during exercise have been reported to be smaller (Pivarnik *et al.*, 1992) or greater (Lehmann and Regnat, 1976). Exercise in pregnancy also results in smaller increases in both venous (Clapp *et al.*, 1987; McMurray *et al.*, 1988; Wolfe *et al.*, 1994a) and arterial (Pivarnik *et al.*, 1992) blood lactate concentrations. Peak blood lactate values are also lower after maximal exercise in pregnancy (McMurray *et al.*, 1991), presumably as a result of dilution of lactate produced in an expanded maternal blood volume (McMurray *et al.*, 1988), fetal lactate utilization as a metabolic fuel (Burd *et al.*, 1975), lowered lactate production, or a combination of these factors (Figure 1). The apparent reduction in maternal buffering capacity is less critical with lower peak blood lactate concentration. However, the hypothetical effects of metabolic acidosis in the maternal system during exercise remain, and are potentially harmful to the fetus. Therefore, information concerning maternal acid-base regulation during strenuous exercise is essential to assess fetal safety.

An increase in fetal $[H^+]$combined with fetal hypoxia due to reduced uterine blood flow (Lotgering *et al.*, 1983), may result in fetal asphyxia (Artal Mittelmark *et al.*, 1991). Asphyxia appears to be more detrimental to the fetus than equivalent degrees of hypoxia (Cohn *et al.*, 1974) and can lead to fetal brain damage and death. Therefore it is important to determine to what extent maternal $[H^+]$ rises during strenuous exercise and if this rise could possibly jeopardize fetal $[H^+]$. Study of the main determinants of maternal plasma $[H^+]$ would also be useful, since this would help to clarify the mechanisms by which the maternal system restores plasma $[H^+]$ to normal resting levels following strenuous exercise.

Stewart's Quantitative Analysis of Acid-Base Balance

The innovative physicochemical approach to acid-base analysis of Peter A. Stewart (1981, 1983) must be considered when investigating acid-base status in human subjects. To date, only one study (Kemp and Wolfe, 1997) has been conducted to validate this approach in the altered physiological conditions of pregnancy.

Stewart's hypothesis examines acid-base equilibria separately in individual fluid compartments (eg., blood plasma). By direct application of fundamental physical and chemical principles, Stewart's analysis of ionic solutions describes the quantitative relationships that determine $[H^+]$, and thus provides a quantitative method of prediction of hydrogen ion activity in human functions. All body

fluid compartments are treated as aqueous solutions, with the following components: (a) water, (b) strong electrolytes, and (c) weak electrolytes (Figure 1). Stewart's quantitative analysis also requires that all systems must behave in accordance with the laws of electroneutrality and conservation of mass, and that all incompletely dissociated substances obey and satisfy dissociation equilibria.
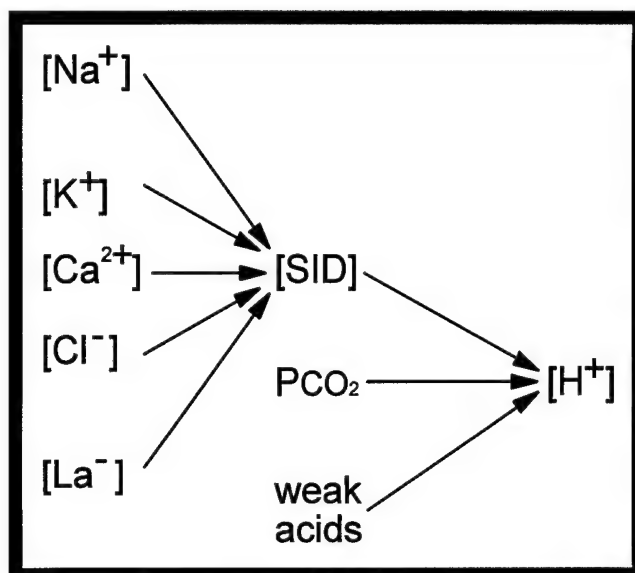
Figure 1   Independent variables that determine [H⁺].

All variables and their quantitative values are defined as either independent or dependent (Stewart, 1981). Three variables can be changed individually and independently in body fluids. These include:

(1)   the carbon dioxide partial pressure (PCO_2);
(2)   the strong ion difference ([SID]), the net strong ion charge (i.e., [SID] = sum of concentrations of all strong cations <u>minus</u> the sum of concentrations of all strong anions);
*   (3)   the total concentrations of all the nonvolatile weak acids, denoted [A_{TOT}].

*   ***Note:*** *Total plasma protein [TP] is the main determinant of [A_{TOT}] in Stewart's physicochemical approach (Stewart, 1981; 1983).*

These three variables are referred to as *independent variables*, with all other variables in the system deemed *dependent variables*. Dependent variables can only be changed if one or more of the *independent variables* change.

By combining the principles of electroneutrality, conservation of mass, and dissociation equilibria relevant to the species in body fluids, equations can be derived to express any dependent variables (namely, [H⁺], [HCO_3^-], [A⁻], [HA], [CO_3^-] and [OH⁻] in terms of all independent variables and dissociation constants (Stewart, 1983). The fourth-order polynomial developed in accordance with these mathematical principles (Stewart, 1983) provides a model that allows the measurement of the independent variables within a system, a meaningful quantitative analysis of their contribution to the system, and calculation of their effect on the dependent variables.

Pregnancy by itself causes a number of changes in the components of Stewart's equation cited above. Both arterial and mixed venous PCO_2 levels are reduced at rest and during submaximal exercise as a result of augmented respiratory sensitivity to carbon dioxide. This effect is mediated by increased circulating progesterone levels and increased hypothalamic progesterone receptors (Bayliss and Milhorn, 1992). The tendency toward respiratory alkalosis is partly offset by renal excretion of bicarbonate. Thus, blood pH rises to approximately 7.46 and buffering capacity $[HCO_3^-]$ is reduced. Changes in plasma electrolytes (primarily $[K^+]$) have also been described during pregnancy (Lucius et al., 1970), and total plasma protein concentration is reduced as a result of maternal blood volume expansion (Pivarnik et al., 1990). Peak plasma lactate values following strenuous exercise also appear to be lower in late gestation vs. the nonpregnant state (Wolfe et al., 1994b).

Physicochemical Approach to Acid-Base Analysis in Human Pregnancy

As described above, pregnancy is accompanied by substantial changes in all three independent variables (PCO_2, [SID], and $[A_{TOT}]$) depicted in Stewart's physicochemical approach to acid-base analysis. A recent study from this laboratory (Kemp et al., 1997) was the first to study acid-base regulation during pregnancy either at rest or during exercise using modern physicochemical principles. Responses of healthy, physically active pregnant (n=9) and nonpregnant (n=14) women were compared at rest and at specific times (1,3,5,7,15 min) during recovery from a progressive maximal cycle ergometer test. As expected, mean values in both groups for venous plasma $[H^+]$, PCO_2, and [TP] increased significantly in the transition from rest to maximal exercise, whereas those for [bicarbonate] and [SID] decreased. However, at rest and during post-exercise recovery, significantly lower mean values were observed in the pregnant group for PCO_2, $[HCO_3^-]$, and [TP]. [SID] was significantly lower in the pregnant group at rest and during early recovery from exercise. Venous plasma $[H^+]$ was always 3-4 nEq/L lower in the pregnant group at all measurement times, but the difference compared to the nonpregnant group revealed statistical significance only in the resting state because of greater variability of values during the post-exercise period. Measured and calculated values for $[H^+]$ were not significantly different from one another, although nonsignificant data trends were observed within both groups to underestimate measured values at rest and to over estimate during post-exercise recovery.

Changes in measured $[H^+]$ were also analyzed during the transition from rest to peak exercise, during early post-exercise recovery (1-7 minutes post-exercise) and in late post-exercise recovery (7-15 minutes post-exercise). Change scores were similar in the pregnant and nonpregnant groups for all three time periods. Analysis of contributions of independent variables to these changes also revealed a similar pattern in both groups. In this regard, reductions in [SID] made substantial % contributions ($\approx$ 60%) to increases in $[H^+]$ in the transition from rest to peak exercise, with smaller contributions caused by increased PCO_2 ($\approx$ 10%) and $[A_{TOT}]$ as reflected by [TP] ($\approx$ 30%). [SID] continued to decrease in early recovery, but this was compensated by substantial reductions in PCO_2 during early recovery, resulting in a modest net reduction in $[H^+]$. Further reductions in PCO_2, increases in [SID] and decreases in $[A_{TOT}]$ (as reflected by [TP]) contributed to reductions in $[H^+]$ in late recovery. In summary, it appears that a lower $[H^+]$ in the resting state in late gestation is the result of reductions in PCO_2 and $[A_{TOT}]$. Changes in $[H^+]$ induced by strenuous exercise and the % contributions of the 3 independent variables to these changes are not altered significantly by pregnancy. Contributions of the independent variables to return $[H^+]$ to resting levels are also similar in the pregnant vs. nonpregnant state.

31

TECHNICAL OBJECTIVES/HYPOTHESES

As described above, an earlier study (Kemp *et al.*, 1997) from this laboratory found significantly lower mean values for PCO_2, [SID] and [TP] in *venous* blood at rest and in recovery from strenuous cycle ergometer exercise in late gestation. In accordance with Stewart's physicochemical approach (1981; 1983), statistical analysis indicated that $\downarrow PCO_2$ and $\downarrow$ [TP] were important factors for the correction of exercise-induced metabolic acidosis during pregnancy and maintenance of [H$^+$] values lower than in the nonpregnant state. We want to conform these findings in *arterialized* blood and also compare the acid-base responses of pregnant women to exercise above and below the ventilatory threshold (Kowalchuk *et al.*, 1994).

Specific statistically testable hypotheses are the following:

♦ Values for $[H^+]_a$ and $[HCO_3^-]_a$ will be significantly lower at rest and during exercise above and below the ventilatory threshold in the pregnant *vs.* nonpregnant state.
♦ Values for P_aCO_2, [SID] and [TP] will be significantly lower at rest and during exercise above and below the ventilatory threshold in the pregnant *vs.* nonpregnant state.
♦ The contribution of P_aCO_2 as an independent determinant of $[H^+]_a$ will be greater in the pregnant *vs.* nonpregnant state at rest and during exercise above and below the ventilatory threshold.
♦ The contribution of $[A_{TOT}]_a$ as reflected by $[TP]_a$ will be significantly greater in the pregnant *vs.* nonpregnant state during exercise above the ventilatory threshold, but not at rest or during exercise below the ventilatory threshold.
♦ Both $[H^+]_a$ and $[HCO_3^-]_a$ will be accurately predicted from P_aCO_2, $[SID]_a$ and $[TP]_a$ at rest and during exercise above and below the ventilatory threshold using the equation of Stewart (1981; 1983).

METHODOLOGY

Subjects

Subjects were 15 healthy, nonsmoking, physically active pregnant women and a control group that included 9 healthy, nonsmoking, physically active nonpregnant women. Prospective subjects responded to newspaper advertisements, posters, flyers, radio programs, and television news programs. Others were recruited from an ongoing physical conditioning study in the Clinical Exercise Physiology Laboratory, Queen's University. The two groups were matched for mean age, height, prepregnant body mass, parity and aerobic fitness. Written informed consent was obtained from all subjects before entry into the study. Medical clearance for each pregnant woman to participate was obtained from the physician or midwife monitoring her pregnancy using a standard form (Wolfe *et al.*, 1989). Final medical clearance was then provided by the medical supervisor of the study (G.A.L. Davies, M.D.). Nonpregnant controls completed the revised Physical Activity Readiness Questionnaire (Thomas *et al.*, 1992).

Experimental Design

Subjects came to the Clinical Exercise Physiology Laboratory on two separate occasions, at least one day apart, for exercise testing. Subjects consumed a standard meal (350 kcal, 40% carbohydrate, 40% fat, 20% protein) 1-2 hours prior to both tests and avoided strenuous physical activity and caffeine on the day of testing. Pregnant subjects were tested between 32-38 weeks of their pregnancy. Control subjects were not using oral contraceptives and their menstrual cycle status at the time of testing was calculated using the first day of their last menstrual cycle and the average length of their cycle.

Exercise Testing Protocol

Basic physical measurements included body height, body mass, the sum of seven skinfolds (Taggart *et al.*, 1967), resting blood pressure and forced vital capacity (Cavitron SC-20A spirometer). Body mass index was calculated as body mass (kg)/body height (m^2). Subjects performed two exercise tests on a Sensor Medics (Model 800S) constant work rate cycle ergometer. Heart rate (HR) was monitored with both a Polar (Vantage model) monitor and a Marquette Max-1 EKG cart. The first test was used to determine the ventilatory anaerobic threshold (T_{VENT}) using the V-slope method (Beaver *et al.*, 1986). The protocol involved 5 minutes of resting data collection and a 4 minute warm-up at 20 watts, followed by a 20 watt/min ramp increase in work rate until a heart rate of at least 170 beats/min was recorded (Lotgering *et al.*, 1992; Kemp *et al.*, 1997). During the test, minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) measured on a breath-by-breath basis using a computerized system that incorporates a respiratory mass spectrometer (Perkin-Elmer, MGA 1100) with a volume turbine (VMM-1100) as described by Hughson *et al.* (1991). Breath-by-breath gas exchange was calculated using the algorithm of Beaver *et al.* (1981). The oxygen pulse ($\dot{V}O_2$/HR) at a heart rate of 170 beats/min was calculated as an index of aerobic working capacity (Wolfe *et al.*, 1994b).

Fetal heart rate was monitored by a qualified obstetric nurse for 20 minutes prior to the graded exercise test described above and for 20 minutes during post-exercise recovery using a Doppler ultrasound (Hewlett-Packard Model 8041-A cardiotocograph). Criteria for the analysis of fetal heart rate were described previously by Webb *et al.* (1994).

The second exercise test involved a period of resting data collection and then a 3 minute warm-up at 0 watts, followed by ramp increases in work rate from 0 watts cycling to a work rate corresponding to 70% or 110% of T_{VENT} over a 30-second time period. Both work rates were continued for approximately 7 minutes after achievement of the prescribed work rate. Subjects rested for 20 minutes between levels.

Biochemistry Analyses

For the first exercise test, venous blood samples were drawn from the antecubital vein, using an indwelling catheter inserted at rest by a registered nurse, at rest and 1, 3 and 5 minutes of post-exercise recovery period. Samples for analysis of lactate concentration ([La$^-$]) were treated with potassium oxalate (antiglycolytic agent) and sodium fluoride (anticoagulant). The samples were then centrifuged for 10 minutes at 2500 rpm and the plasma frozen for later analysis. Plasma samples were analyzed for determination of [La$^-$] using an automated analyzer (Yellow Springs Instruments, Model 23-L). The analyzer was calibrated before analysis using 5 and 15 mmol/L standards and at regular intervals during the analysis. The test-retest reliability of measurements was described in an earlier publication from this laboratory (Wolfe et al., 1994b).

The second exercise test involved the insertion of an indwelling catheter into a dorsal hand vein situated as far from the thumb as possible. The hand and lower arm were soaked in a warm water bath and then wrapped in a heating pad to promote vasodilation. Arterialized blood samples were then collected at rest and during the sixth minute of exercise at the two work rates corresponding to 70% and 110% of T_{VENT}.

Blood samples for the determination of oxygen tension (PO$_2$), PCO$_2$, [HCO$_3^-$] and [H$^+$] were collected in a syringe containing lyophilized heparin and analyzed immediately using a Radiometer ABL 30 acid-base analyzer at a standard temperature of 37°C. Quality control using four control liquids was done on testing days to ensure the analyzer was functioning properly.

The remaining blood was then centrifuged for 10 minutes at 2500 rpm and frozen for later analysis of [TP], [ALB] and electrolytes. [TP] was measured using the biuret method (Young et al., 1975a). Total weak acid ([A$_{TOT}$] mEq/L) was calculated from [TP] (g/L) to mEq/L using the conversion factor 0.243 (Kowalchuk and Scheuermann, 1993; Van Slyke et al., 1928). [ALB] was determined using a conventional dye-binding method (Doumas et al., 1975). [Pi$_{TOT}$] was determined using phosphomolybdate complex. Plasma concentrations of sodium ([Na$^+$]), potassium ([K$^+$]) and chloride ([Cl$^-$]) were analyzed using ion-selective electrodes. Total plasma calcium ([Ca$^{++}_{TOT}$]) was measured using a timed endpoint method (Michaylova, 1971). Values for ionized calcium concentration ([Ca^{2+}]) were then calculated as: 0.469 ([Ca$^{2+}_{TOT}$] + 0.02 [43-[ALB]]). [La$^-$] was determined as described above and the strong ion difference ([SID]) expressed in nEq/L was calculated as: ([Na$^+$] + [K$^+$] + [Ca^{2+}]) - ([Cl$^-$] + [La$^-$]). Globulin concentration ([GLOB]) was calculated by subtracting the [ALB] from [TP] so that the A/G ratio could be determined. The interassay coefficient of variability was less than 3% for all of the procedures listed above.

Osmolality was determined using an automated analyzer (Osmette A, Model #5002, Precision Systems Inc.) that utilizes the freezing point depression technique.

Stewart's Quantitative Analyses

The calculation of $[H^+]$ from PCO_2, [SID] and total weak acid was done using the Stewart model (Stewart, 1981; 1983) using the following equation:

$$[H^+]^4 + (K_A + [SID]) [H^+]^3$$
$$\div \{K_A ([SID] - [A_{TOT}])$$
$$- (K_C \times PCO_2 + K'_w)\} [H^+]^2$$
$$- \{K_A (K_C \times PCO_2 + K'_w)$$
$$\div (K_3 \times K_C \times PCO_2)\} [H^+]$$
$$- (K_A \times K_3 \times K_C \times PCO_2) = 0$$

where

$$K'_w = 4.4 \times 10^{-14} \text{ (eq/l)}^2; K_c$$

$$= 2.46 \times 10^{-11} \text{ (eq/l)}^2/\text{Torr}; K_3 = 6.0 \times 10^{-11} \text{ (eq/l)};$$
$$K_A = 3.0 \times 10^{-7} \text{ (eq/l); [SID]} = ([Na^+] + [K^+]) - ([Cl^-] \div [La^-])$$

where K'_w, K_A and K_c and K_3 are the equilibrium constants for the ion product of water, the weak acid system, and the carbonic acid system, respectively.

Stewart's equation was also used to estimate the contribution of the three independent variables to differences observed between groups at rest and to changes in $[H^+]$ during the transition from rest to exercise at 70% T_{VENT} and from rest to exercise at 110% T_{VENT}. Differences for absolute and percent contributions for each independent variable between groups during these time intervals were analyzed by using a two-way analysis of variance (pregnant/control *vs.* time) with repeated measures. Results of all statistical tests were considered significant if $P < 0.05$. When a significant between group main effect was observed, independent *t*-statistics were used to identify significant differences between group means at the two time intervals.

Statistical Analyses

Physical characteristics and responses to graded exercise tests were compared using Student's *t*-statistic for independent samples. Data at rest and during exercise at 70% and 110% T_{VENT} were compared within and between subjects using a two-way analysis of variance (groups *vs.* rest/exercise level) with repeated measures on the second factor. When a significant ($p \leq 0.05$) between group main effect was observed, separate independent *t*-statistics were used to identify significant differences between group means at rest, at 70% T_{VENT} and at 110% T_{VENT}. To reduce the likelihood of Type 1 statistical error, differences were considered significant if $p \leq 0.017$ (calculated as 0.05 divided by 3 comparisons).

When a significant ($p \leq 0.05$) within subjects main effect was observed, simple within subjects contrasts were used to detect significant differences between rest, 70% T_{VENT} and 110% T_{VENT}. Again to reduce the possibility of Type 1 error, changes from rest to 70% or 110% T_{VENT} or from 70% to 110% T_{VENT} were considered significant if $p \leq 0.017$. If no significant interaction was observed for within and between subjects main effects, it was assumed that changes were significant within both groups. When a significant within vs. between subjects interaction was observed, a separate one-way analysis of variance with repeated measures was conducted within each group. When a significant within subject effect was observed ($p \leq 0.05$), simple within subjects contrasts were used to detect significant ($p \leq 0.017$) differences between rest and the two exercise levels within each group.

Measured [H^+] values and those calculated using the Stewart equation were compared within and between groups using a two-way analysis of variance (group vs. measured/calculated values) with repeated measures on the second factor. When a significant ($p \leq 0.05$) between group main effect was observed, independent t-statistics were used to identify significant differences between group means at rest and at both 70% and 110% T_{VENT}. Results were considered significant if $p \leq 0.017$. Since a statistically significant ($p \leq 0.05$) interaction was observed between within group and between group main effects, separate one-way analyses of variance with repeated measures were conducted within each group to detect significant ($p \leq 0.017$) differences among measured [H^+] values and those calculated using the Stewart equation. If a significant F-ratio was observed, simple within subjects contrasts were used to identify significant differences between paired means. Results were considered significant if $p \leq 0.017$.

Pearson correlation coefficients were calculated to measure the strength of the relationships between measured [H^+] and those [H^+] calculated from the Stewart model.

RESULTS

Subject Characteristics

Subjects in both groups were aged between 20 and 40 years. Mean values were 29.3 ± 0.8 years and 28.3 ± 2.3 years for the pregnant group and nonpregnant group, respectively. Mean gestational age of the pregnant group was 37 ± 0.3 weeks. As expected, body mass and BMI were significantly higher in the pregnant group compared to the nonpregnant group at the time of testing. The between group difference for the sum of 7 skinfolds also approached statistical significance. There were no significant differences in mean age, body height, parity, T_{VENT} and O_2 pulse at 170 bpm. The work rate at 170 beats/min during the graded cycling test was significantly higher in the control group vs. pregnant group.

Table 1   **Physical Characteristics of Subjects**

Variable	Pregnant Group (n = 15)	Control Group (n = 9)
Age (years)	29.3 ± 0.8	28.3 ± 2.3
Gestational Age (weeks)	37.0 ± 0.3	n/a
Height (cm)	163.2 ± 2.1	162.3 ± 1.7
Body Mass (kg)	77.6 ± 2.8^a	63.1 ± 2.9
Body Mass Index	29.1 ± 0.8^a	23.9 ± 0.9
Prepregnancy Body Mass (kg)	63.8 ± 2.8	n/a
Prepregnant Body Mass Index	23.9 ± 0.9	n/a
Sum of Seven Skinfolds (mm)	134 ± 12	102 ± 11
Parity	0.5 ± 0.2	0.6 ± 0.2
$\dot{V}O_2$ @ T_{VENT}	1.69 ± 0.08	1.82 ± 0.03
O_2 Pulse @ 170 beats/min ($\dot{V}O_2$/HR)	11.8 ± 0.6	13.6 ± 0.6
Watts @ 170 beats/min	162 ± 8^a	200 ± 7

Values are means $\pm$ SE.
[a]Significant difference ($p \leq 0.05$) between groups.

Metabolic and Cardiorespiratory Responses

Responses at rest and during cycling at 70% T_{VENT} and 110% T_{VENT} appear in Tables 2a-c. Mean values for HR, ($\dot{V}_E$), tidal volume (V_T), breathing frequency (f), $\dot{V}O_2$, $\dot{V}CO_2$ and the respiratory exchange ratio (RER) increased significantly from rest to cycling at 70% T_{VENT} and from cycling at 70% T_{VENT} to cycling at 110% T_{VENT} in both groups.

Within both groups, the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) increased significantly from 70% T_{VENT} to 110% T_{VENT} whereas the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) decreased significantly from rest to both 70% T_{VENT} and 110% T_{VENT} and increased between 70% and 110% T_{VENT}. End tidal oxygen tension ($P_{ET}O_2$) increased significantly in the transition from rest to both 70% and 110% T_{VENT} and decreased between 70% T_{VENT} and 110% T_{VENT}. End tidal carbon dioxide tension ($P_{ET}CO_2$) increased significantly from rest to both 70% and 110% T_{VENT} and decreased significantly between 70% and 110% T_{VENT}. Calculated P_aCO_2 increased from rest to 70% T_{VENT} and decreased significantly from 70% to 110% T_{VENT} within both groups.

Similar values were observed in both groups for $\dot{V}_E$, V_T, $\dot{V}O_2$, $\dot{V}CO_2$, and RER at rest and both exercise levels. However, HR and f were significantly greater in the pregnant *vs.* control group at rest. $\dot{V}_E/\dot{V}O_2$ was greater in the pregnant *vs.* control group at rest and 70% T_{VENT}. $\dot{V}_E/\dot{V}CO_2$ was greater in the pregnant *vs.* control group at level 2 and 3. $P_{ET}CO_2$ and calculated P_aCO_2 were significantly less in the pregnant *vs.* control group under all experimental conditions.

Table 2a   Resting Measurements

Variable	Pregnant Group (n = 15)	Control Group (n = 9)
Heart Rate (beats/min)	89 ± 2[a]	71 ± 3
$\dot{V}_E$ (L/min)	11.1 ± 0.4	8.1 ± 0.6
V_T (litres)	0.60 ± 0.02	0.56 ± 0.05
f (breaths/min)	19.2 ± 0.8[a]	15.8 ± 1.1
$\dot{V}O_2$ (L/min)	0.38 ± 0.01	0.34 ± 0.01
$\dot{V}CO_2$ (L/min)	0.33 ± 0.01	0.29 ± 0.01
RER	0.89 ± 0.02	0.84 ± 0.02
$\dot{V}_E/\dot{V}O_2$	30.4 ± 1.2[a]	24.5 ± 1.4
$\dot{V}_E/\dot{V}CO_2$	33.9 ± 1.2	30.5 ± 1.7
$P_{ET}O_2$ (mmHg)	113.6 ± 0.6[a]	106.7 ± 1.6
$P_{ET}CO_2$ (mmHg)	31.8 ± 0.6[a]	37.6 ± 0.7
Calculated $PaCO_2$ (mmHg)	32.9 ± 0.5[a]	38.1 ± 0.7

Values are means $\pm$ SE.
[a]Significant difference ($p \leq 0.017$) between groups at rest.

Table 2b   **Responses to Upright Cycling at 70% T_{VENT}**

Variable	Pregnant Group (n = 15)	Control Group (n = 9)
Heart Rate (beats/min)	131 ± 3^b	122 ± 6
$\dot{V}_E$ (L/min)	37.1 ± 2.1^b	32.1 ± 1.9
V_T (litres)	1.35 ± 0.08^b	1.36 ± 0.06
f (breaths/min)	28.2 ± 1.2^b	24.0 ± 1.2
$\dot{V}O_2$ (L/min)	1.29 ± 0.07^b	1.37 ± 0.05
$\dot{V}CO_2$ (L/min)	1.22 ± 0.07^b	1.31 ± 0.06
RER	0.95 ± 0.01^b	0.96 ± 0.01
$\dot{V}_E/\dot{V}O_2$	29.2 ± 1.2^a	23.6 ± 0.8
$\dot{V}_E/\dot{V}CO_2$	$30.6 \pm 1.0^{a,b}$	24.5 ± 0.5
$P_{ET}O_2$ (mmHg)	$110.9 \pm 1.1^{a,b}$	105.0 ± 1.4
$P_{ET}CO_2$ (mmHg)	$34.4 \pm 0.8^{a,b}$	41.4 ± 0.9
Calculated $PaCO_2$ (mmHg)	$33.7 \pm 0.7^{a,b}$	40.0 ± 0.8

Values are means ± SE.
[a]Significant difference ($p \leq 0.017$) between groups at 70% T_{VENT}.
[b]Significant change ($p \leq 0.017$) within groups from Rest to cycling at 70% T_{VENT}.

Table 2c   **Responses to Upright Cycling at 110% T_{VENT}**

Variable	Pregnant Group (n = 15)	Control Group (n = 9)
Heart Rate (beats/min)	$163 \pm 3^{b,c}$	153 ± 6
$\dot{V}_E$ (L/min)	$60.5 \pm 2.8^{b,c}$	56.0 ± 2.8
V_T (litres)	$1.86 \pm 0.10^{b,c}$	1.93 ± 0.08
f (breaths/min)	$33.5 \pm 1.8^{b,c}$	29.5 ± 1.3
$\dot{V}O_2$ (L/min)	$1.92 \pm 0.11^{b,c}$	2.06 ± 0.05
$\dot{V}CO_2$ (L/min)	$1.97 \pm 0.11^{b,c}$	2.11 ± 0.06
RER	$1.03 \pm 0.01^{b,c}$	1.02 ± 0.01
$\dot{V}_E/\dot{V}O_2$	32.1 ± 1.3^{c}	27.5 ± 0.8
$\dot{V}_E/\dot{V}CO_2$	$31.2 \pm 1.1^{a,b,c}$	26.7 ± 0.6
$P_{ET}O_2$ (mmHg)	113.3 ± 1.1^{c}	109.0 ± 1.4
$P_{ET}CO_2$ (mmHg)	$34.1 \pm 0.9^{a,b,c}$	39.5 ± 0.9
Calculated $PaCO_2$ (mmHg)	$32.3 \pm 0.7^{a,c}$	37.0 ± 0.8

Values are means ± SE.
[a]Significant difference ($p \leq 0.017$) between groups cycling at 110% T_{VENT}.
[b]Significant change ($p \leq 0.017$) within group from Rest to cycling at 110% T_{VENT}.
[c]Significant change ($p \leq 0.017$) within group from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.

Dependent Acid-Base Variables

During all three experimental conditions [H$^+$] and [HCO$_3^-$] were lower in the pregnant *vs.* control group (Table 3). Results were statistically significant except for [H$^+$] at 110% T_{VENT} where a trend that approached significance was observed. In both groups, [H$^+$] increased in the transition from rest to 70% T_{VENT} and from 70% T_{VENT} to 110% T_{VENT}. [HCO$_3^-$] was significantly lower at 110% T_{VENT} compared to rest and 70% T_{VENT}.

Changes in measured [H$^+$] from rest to 70% T_{VENT} and rest to 110% T_{VENT} did not differ significantly between groups (Figure 2). In both groups, a small (1.4 - 1.8 neq/L) increase was observed in the transition from rest to 70% T_{VENT} and a larger (4.5 - 5.3 neq/L, P < 0.05) increase was observed from rest to 110% T_{VENT}.

Table 3   **Measured [H⁺] and Calculated [HCO₃⁻] at Rest and at Two Work Rates**

Variable	$[H^+]$ (nEq/L)	$[HCO_3^-]$ (mEq/L)
REST		
PG	37.13 ± 0.32^a	21.5 ± 0.4^a
CG	40.07 ± 0.53	24.3 ± 0.5
70% T_{VENT}		
PG	$38.55 \pm 0.45^{a,b}$	21.4 ± 0.3^a
CG	41.88 ± 0.71	23.6 ± 0.6
110% T_{VENT}		
PG	$42.45 \pm 0.80^{b,c}$	$19.0 \pm 0.3^{a,b,c}$
CG	44.60 ± 1.01	21.0 ± 0.8

Values are means ± SE.
PG - Pregnant Group (n=15); CG - Control Group (n=9).
[a]Significant difference ($p \le 0.017$) between groups.
[b]Significant change ($p \le 0.017$) within group from Rest.
[c]Significant change ($p \le 0.017$) within group from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.
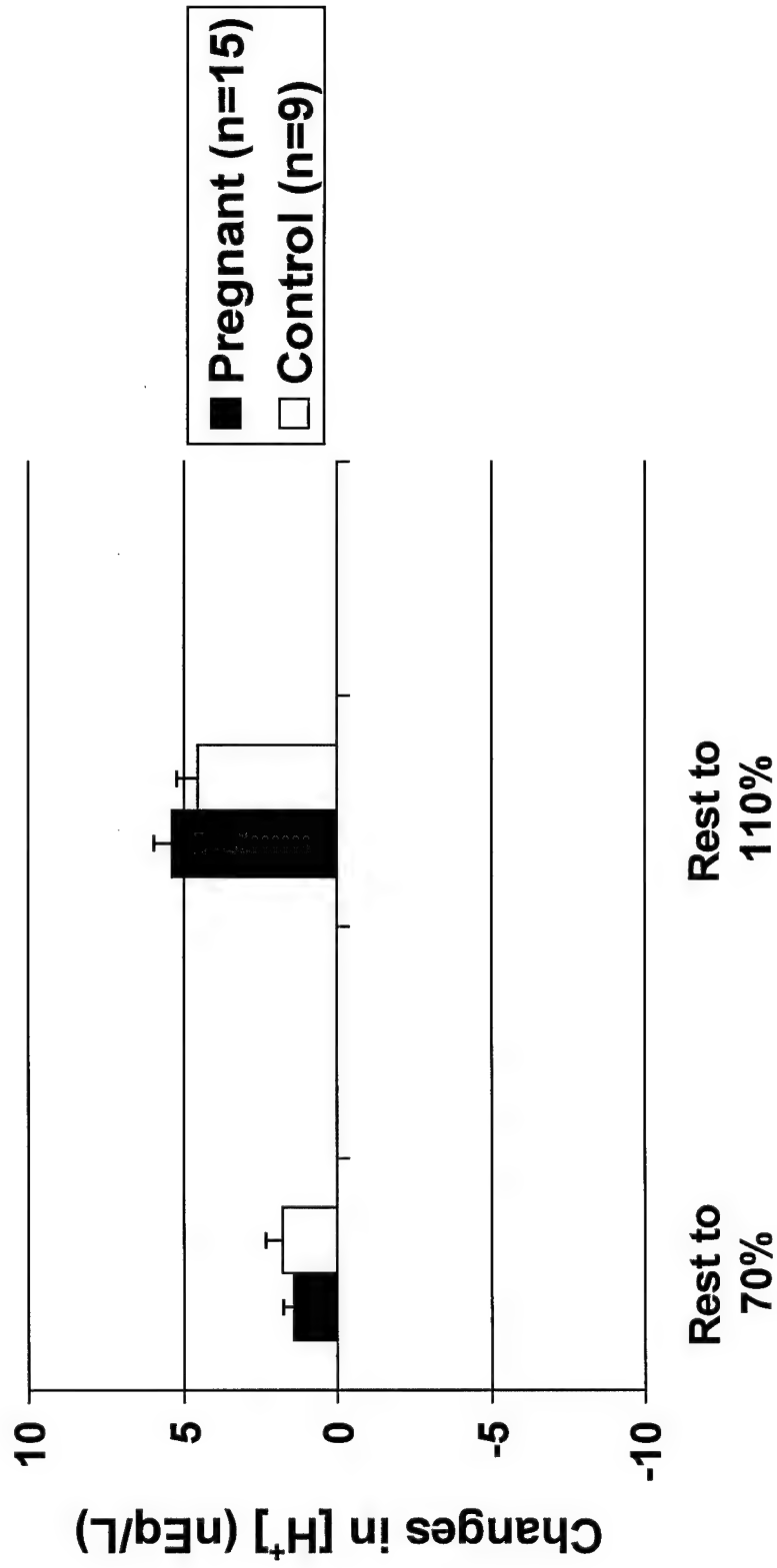
Figure 2   Changes in Measured [H+]

Independent Acid-Base Variables

PCO_2 was significantly lower in the pregnant *vs.* control group at all three activity levels (Figure 2). Within groups PCO_2 did not change significantly in the transition from rest to 70% T_{VENT} or from rest to 100% T_{VENT}, but decreased significantly from 70% to 110% T_{VENT}. PCO_2 appears in Figure 2.

Both $[Na^+]$ and $[K^+]$ increased in the transition from rest to both 70% and 110% T_{VENT} and from 70% T_{VENT} to 110% T_{VENT}. $[Na^+]$ in the pregnant group was significantly lower at rest and both exercise levels compared to the control group. As expected, values for plasma osmolality changed in parallel with those for $[Na^+]$. The pregnant group $[K^+]$ was significantly lower at rest and 70% T_{VENT} compared to the control group. $[Ca^{++}]$ was greater at 110% T_{VENT} in both groups than at rest and 70% T_{VENT}. The pregnant group's values for $[Ca^{2+}]$ were significantly higher than those of the control group at 110% T_{VENT}. Mean values were also significantly higher in the pregnant *vs.* control groups for $[Ca^{2+}]$ at 70% T_{VENT}. There were no differences in $[Cl^-]$ between groups at any level. $[Cl^-]$ increased significantly in the transition from rest to 70% T_{VENT}. $[La^-]$ increased significantly from rest to 70% T_{VENT} and from 70% T_{VENT} to 110% T_{VENT} as expected. There were no between group differences in $[La^-]$.

The calculated [SID] was significantly lower in the pregnant group *vs.* control group at rest and 70% T_{VENT} but not at 110% T_{VENT} (Figure 3). Within groups [SID] decreased significantly in the transition from rest to 110% T_{VENT}. Calculated values for [SID] appear in Figure 4.

^{a}Significant difference ($p \le 0.017$) between groups.

^{b}Significant change ($p \le 0.017$) within group from Rest.

^{c}Significant change ($p \le 0.017$) within group from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.
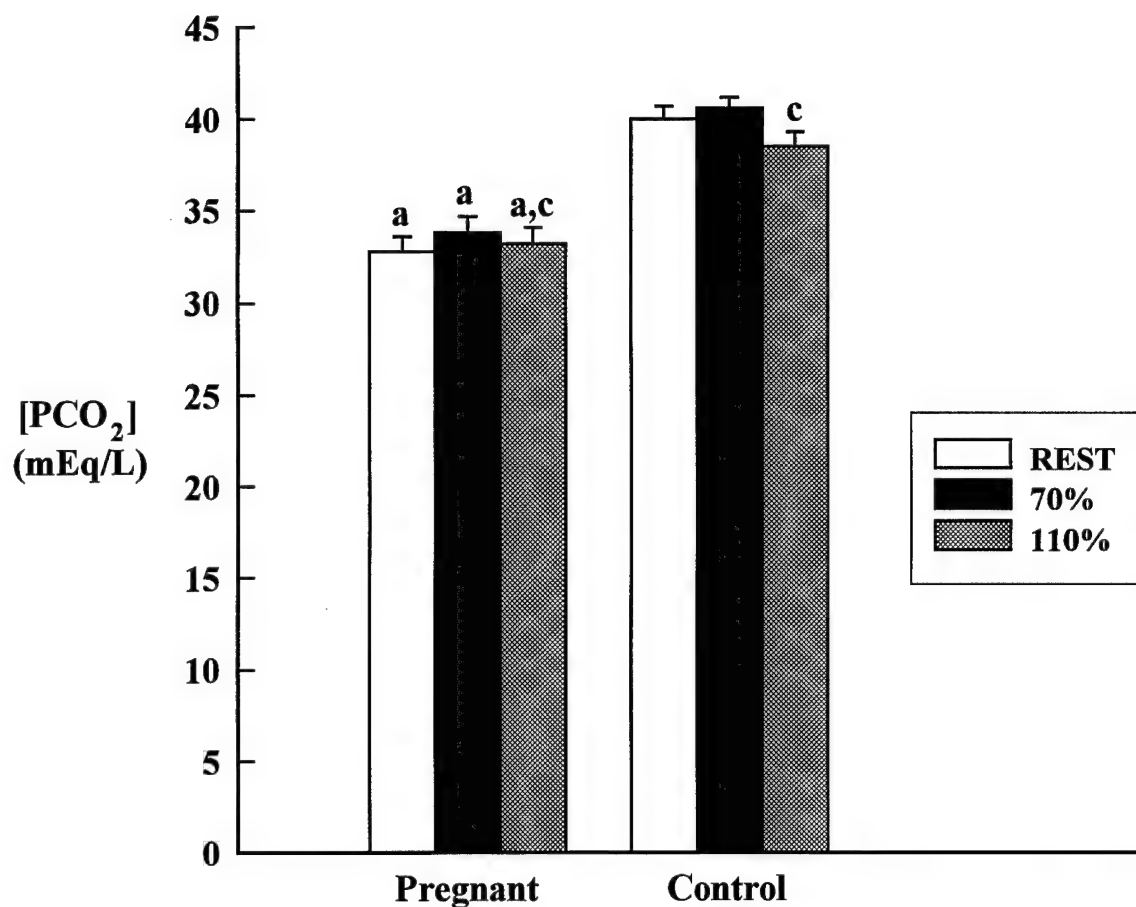
Figure 3   Arterialized Plasma PCO$_2$ at Rest and at Two Work Rates.

Table 4   Plasma Strong Ions and Osmolality at Rest and at Two Work Rates

Variable	$[Na^+]$ (mEq/L)	OSMO (mOsm/kg H_2O)	$[K^+]$ (mEq/L)	$[Ca^{++}_{TOT}]$ (mmol/L)	Ionized $[Ca^{++}]$ (mEq/L)	$[Cl^-]$ (mEq/L)	$[La^-]$ (mEq/L)
REST							
PG	136 ± 0.3^a	276 ± 0.6^a	3.9 ± 0.0^a	2.23 ± 0.4^a	2.34 ± 0.04	103 ± 0.5	1.4 ± 0.1
CG	139 ± 0.6	284 ± 1.0	4.3 ± 0.0	2.44 ± 0.04	2.28 ± 0.04	104 ± 0.6	1.2 ± 0.3
70% T_{VENT}							
PG	$137 \pm 0.4^{a,b}$	$280 \pm 0.7^{a,b}$	$4.5 \pm 0.06^{a,b}$	2.33 ± 0.03	2.38 ± 0.02^a	104 ± 0.5^b	2.2 ± 0.2^b
CG	140 ± 0.6	287 ± 0.8	4.8 ± 0.05	2.43 ± 0.04	2.24 ± 0.02	105 ± 0.6	2.6 ± 0.6
110% T_{VENT}							
PG	$138 \pm 0.5^{a,b,c}$	$283 \pm 0.9^{a,b,c}$	$4.8 \pm 0.1^{b,c}$	$2.40 \pm 0.03^{a,b,c}$	$2.44 \pm 0.02^{a,b,c}$	104 ± 0.5	$5.4 \pm 0.4^{b,c}$
CG	141 ± 0.7	291 ± 1.3	5.0 ± 0.1	2.53 ± 0.03	2.30 ± 0.02	105 ± 0.7	5.8 ± 0.9

Values are means ± SE.

PG - Pregnant Group (n=15); GC - Control Group (n=9).

[a]Significant difference ($p \leq 0.017$) between groups.

[b]Significant change ($p \leq 0.017$) within groups from Rest.

[c]Significant change ($p \leq 0.017$) within groups from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.

46
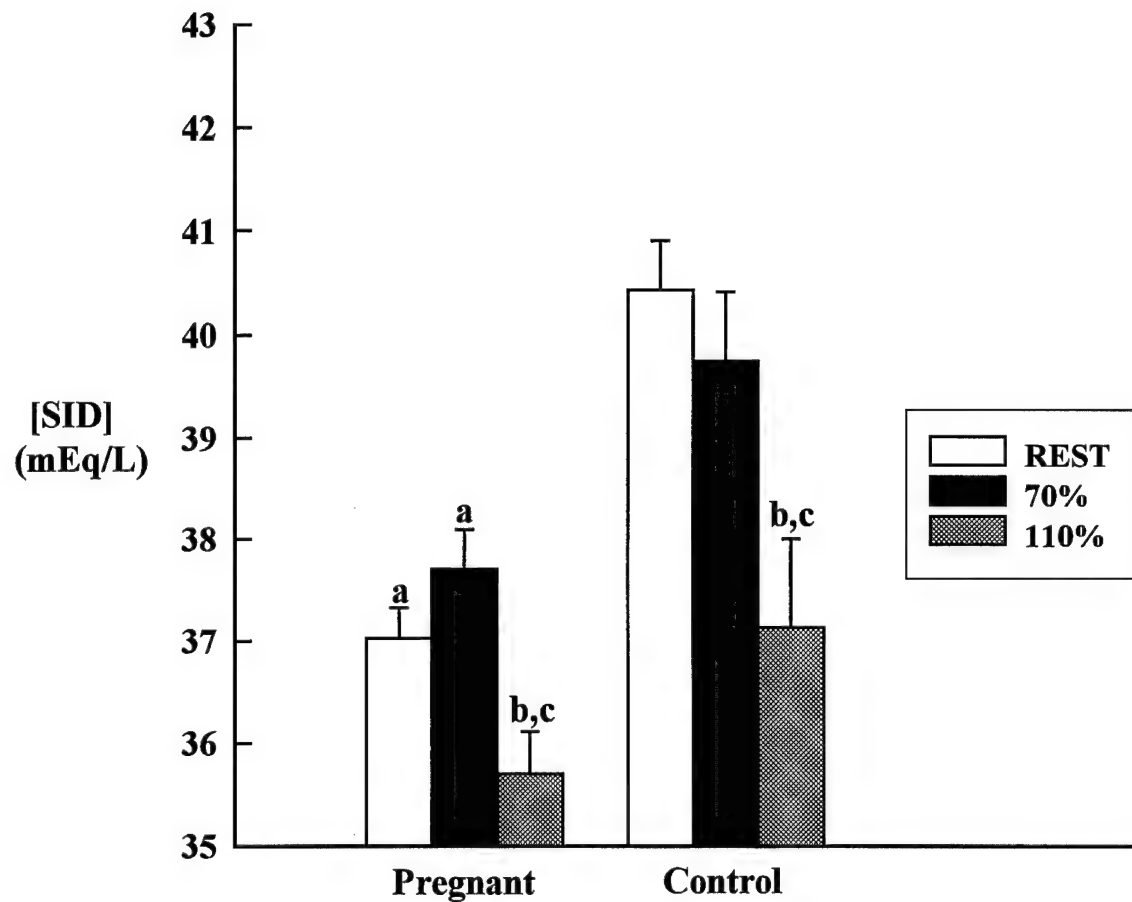
^{a}Significant difference ($p \leq 0.017$) between groups.

^{b}Significant change ($p \leq 0.017$) within group from Rest.

^{c}Significant change ($p \leq 0.017$) within group from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.

Figure 4   *Calculated Plasma [SID] at Rest and at Two Work Rates.*

47

For both groups [TP], [ALB] and [Pi_{TOT}] increased significantly in the transition from rest to both 70% T_{VENT} and 110% T_{VENT}. [ALB] also increased significantly from 70% to 110% T_{VENT}. There were no significant changes across measurement conditions for [GLOB] and A/G ratio. At rest, 70% T_{VENT} and 110% T_{VENT} the values for [TP], [ALB] and the A/G ratio were significantly lower in the pregnant group compared to the nonpregnant group. The values for [GLOB] and [Pi_{TOT}] did not differ between groups at any activity level. Calculated values for [A_{TOT}] appear in Figure 5.

Table 5 **Plasma Protein and Total Phosphate at Rest and During Two Work Rates**

| Variable	[TP] (g/L)	[ALB] (g/L)	[GLOB] (g/L)	A/G	[Pi_{TOT}] (mmol/L)
REST					
PG	64 ± 1^a	30 ± 0.5^a	33 ± 1.2	0.92 ± 0.04^a	1.11 ± 0.04
CG	74 ± 2	43 ± 0.8	29 ± 2.2	1.53 ± 0.10	1.22 ± 0.07
70% T_{VENT}					
PG	$66 \pm 1^{a,b}$	$32 \pm 0.6^{a,b}$	35 ± 0.7	0.92 ± 0.02^a	1.22 ± 0.04^b
CG	78 ± 2	45 ± 0.6	33 ± 1.7	1.39 ± 0.06	1.29 ± 0.07
110% T_{VENT}					
PG	$69 \pm 1^{a,b}$	$33 \pm 0.7^{a,b,c}$	36 ± 1.0	0.92 ± 0.03^a	$1.30 \pm 0.03^{b,c}$
CG	79 ± 1	47 ± 0.4	33 ± 1.6	1.43 ± 0.08	1.46 ± 0.06

Values are means ± SE.

PG - Pregnant Group (n=15); CG - Control Group (n=9)

[a]Significant difference (p≤0.017) between groups.

[b]Significant change (p≤0.017) within groups from Rest.

[c]Significant change (p≤0.017) within groups from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.
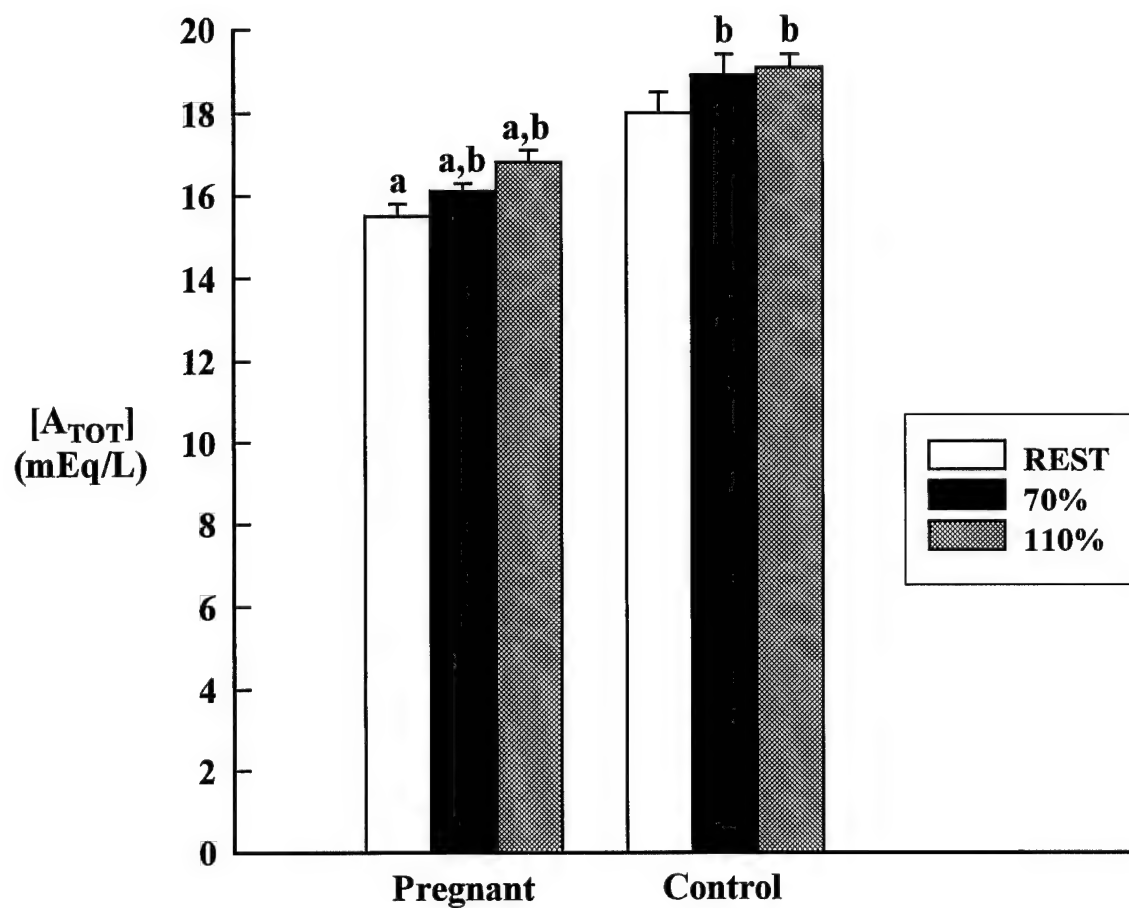
[a]Significant difference ($p \leq 0.017$) between groups.

[b]Significant change ($p \leq 0.017$) within group from Rest.

[c]Significant change ($p \leq 0.017$) within group from cycling at 70% T_{VENT} to cycling at 110% T_{VENT}.

Figure 5   *[A$_{TOT}$] as Reflected by [TP] at Rest and at Two Work Rates.*

Calculations of [H⁺] Using the Stewart Model

The $[H^+]$ calculated using the Stewart model (Table 6) was significantly different from the measured $[H^+]$ at rest, 70% T_{VENT} and 110% T_{VENT} in the pregnant group but not in the control group. At all three measurement times the calculated $[H^+]$ was significantly lower than the measured $[H^+]$ in the pregnant group. The $[H^+]$ calculations in the control group exhibited a nonsignificant trend to overestimate $[H^+]$. Measured $[H^+]$ and calculated $[H^+]$, using the Stewart model, were significantly ($P < 0.05$) correlated at all measurement except at rest in the control group.

Calculations of Contributions of PCO_2, [SID] and $[A_{TOT}]$ to Changes in $[H^+]$

Utilizing the Stewart equation, the contributions of the independent variables to changes in $[H^+]$ were calculated (Figure 6). In the pregnant group during the transition from rest to the 70% T_{VENT} work rate the increase in $[H^+]$ was mainly the result of an increase in PCO_2 and $[A_{TOT}]$ while an increased [SID] attenuated this response. The increase in $[H^+]$ in the control group during the transition from rest to the 70% T_{VENT} work rate was mainly due to an increased $[A_{TOT}]$ and a decreased [SID], with an increased PCO_2 contributing slightly. During the transition from rest to the 110% T_{VENT} workload the increase in $[H^+]$ was mainly due to an increased $[A_{TOT}]$ and decreased [SID] in both groups. At the 110% workload the increased PCO_2 in the pregnant group made a small contribution to the rise in $[H^+]$, while in the control group a lowered PCO_2 slightly attenuated the rise in $[H^+]$.

Table 6   **Values of the Measured [H⁺] and Predicted [H⁺], Using the Stewart Model at Rest and the Two Work Rates**

Variable	Measured [H$^+$] (nEq/L)	*Stewart Model* Predicted [H$^+$] (nEq/L)	Mean Difference (nEq/L)	r
REST				
PG	37.13 ± 0.32	34.91 ± 0.75[a]	-2.22	+0.72
CG	40.07 ± 0.53	40.18 ± 0.97	+0.11	-0.01
70% T$_{VENT}$ workload				
PG	38.55 ± 0.45	35.82 ± 0.76[a]	-2.73	+0.86
CG	41.88 ± 0.71	43.48 ± 1.6	+1.60	+0.73
110% T$_{VENT}$ workload				
PG	42.45 ± 0.80	39.42 ± 1.21[a]	-3.03	+0.79
CG	44.60 ± 1.01	46.56 ± 1.44	+1.96	+0.81

Values are means ± SE.

PG - Pregnant Group (n=15); CG - Control Group (n=9)

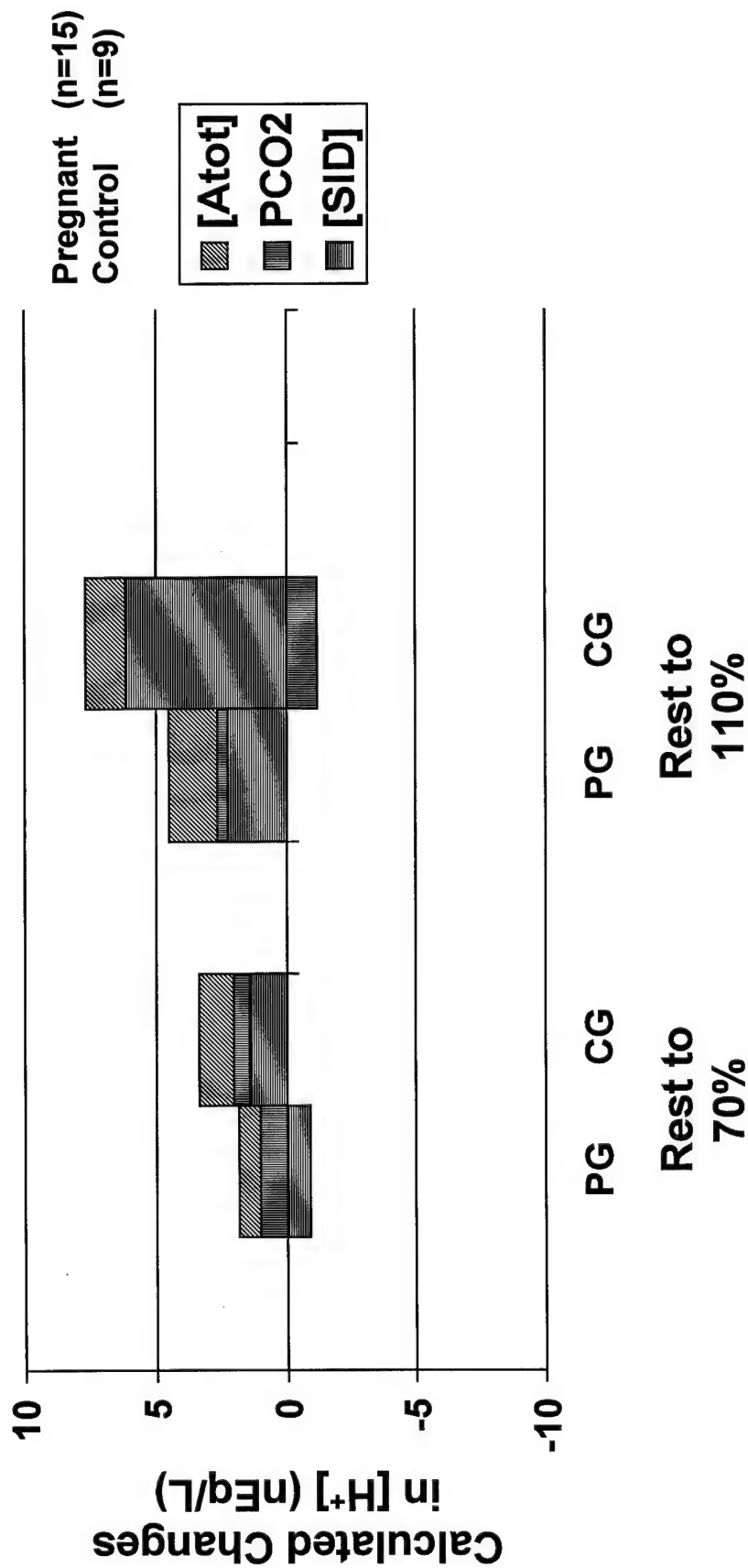[a]Significant difference (p≤0.017) between measured and predicted [H$^+$] values.

Figure 6 Calculated Contributions of Independent Variables to Changes in [H+]

DISCUSSION

This is only the second study to employ modern physicochemical principles for this purpose. This study was conducted to compare mechanisms of acid-base regulation in arterialized blood of healthy, physically active pregnant and nonpregnant subjects at rest and during exercise above and below the ventilatory anaerobic threshold.

Generalized metabolic and cardiorespiratory responses at rest and during both exercise tests and differences between groups were consistent with previous studies of exercising pregnant women. Although between group differences did not always reach statistical significance, it is well documented that respiratory sensitivity to CO_2 is increased, resulting in higher values for $\dot{V}_E$, $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ as well as an increased $P_{ET}O_2$ and reduced $P_{ET}CO_2$ both at rest and during submaximal exercise relative to the nonpregnant state (Knuttgen and Emerson, 1974; Pernoll et al., 1975; Ohtake and Wolfe, in press). The observation that $\dot{V}_E$ at the two exercise intensities, wasn't significantly higher in the pregnant group compared to the nonpregnant group is probably a result of the slightly lower $\dot{V}O_2$ values in the pregnant group compared to those in the nonpregnant group. Since pulmonary ventilation is driven by metabolic rate, it seems likely that $\dot{V}_E$ would have been significantly higher in the pregnant group if the values for the $\dot{V}O_2$ during the exercise bouts had been perfectly equated across groups. RER does not appear to be altered at work rates below the onset of respiratory compensation for metabolic acidosis (Wolfe et al., 1994b; Lotgering et al., 1995) and it is well documented that HR is augmented both at rest and during submaximal exercise (Sady et al., 1989; Ohtake and Wolfe, in press). Finally, reported effects of pregnancy on maximal aerobic power have been variable (Wolfe et al., 1989; Wolfe and Mottola, 1993) and existing studies indicate that T_{VENT} is not changed (Wolfe et al., 1994b; Lotgering et al., 1995). Thus, our results are consistent with and confirm the results of earlier studies of the effects of human pregnancy on metabolic and cardiorespiratory responses to exercise.

Measured $[H^+]$ values in the pregnant group were lower compared to the control groups at rest and at both exercise levels. Differences were statistically significant at rest and 70% T_{VENT}, but significance was lost at 110% T_{VENT}. The rise in $[H^+]$ from rest to 70% T_{VENT} and rest to 110% T_{VENT} was similar in both groups. These findings are similar to those of Kemp et al. (1997) and Pivarnik et al. (1992), but differ from those of Lehmann and Regnat (1976) who observed larger reductions in pH in the pregnant group compared to the control group in the transition from rest to submaximal exercise. Although $[H^+]$ values within the pregnant group were not significantly lower than those of the control group at 110% T_{VENT}, a trend for lower values was apparent and should become significant with a larger sample size. The higher $[H^+]$ in the pregnant group compared to the nonpregnant group during exercise in the study by Lehmann and Regnat (1976) is at odds with the findings of Kemp et al. (1997), Pivarnik et al. (1992) and the present study and thus, may not be an accurate reflection of the normal response of pregnant women to exercise.

Values for $[HCO_3^-]$ were lower in the pregnant group vs. the control group at rest and both exercise intensities. Only the 110% T_{VENT} work rate caused a significant decrease in $[HCO_3^-]$ which did not differ between groups. Once again, these results differ from those of Lehmann and Regnat (1976) who found that exercise-induced decreases in $[HCO_3^-]$ were greater in pregnancy.

Arterialized PCO_2 values were in close agreement with the calculated P_aCO_2 values. This supports the hypothesis that P_aCO_2 can be accurately predicted from $P_{ET}CO_2$ and V_T using the equation of Jones et al. (1979). Arterialized PCO_2 values in the pregnant group were significantly lower than those of the control group under all experimental conditions. This is the result of pregnancy-induced increases in respiratory sensitivity to carbon dioxide (Lyons and Antonio, 1959; Bayliss and Millhorn, 1992). Augmented respiratory sensitivity in pregnancy has traditionally been attributed to the effects of increased levels of progesterone, a known respiratory stimulant (Lyons and Antonio, 1959; Machida, 1981; Brodeur et al., 1986) and estrogen. The increased estrogen levels elevates the number of progesterone receptors in the hypothalamus (MacLusky and McEwan, 1978). In accordance with recent findings of Jennings and associates (Jennings, 1994), the increase in $\dot{V}_E$ in pregnancy may also be due to changes in osmolality, [SID] and angiotensin II levels which have been implicated in the control of ventilation. During pregnancy osmolality decreases (Duvekot et al., 1993), [SID] decreases (Kemp et al., 1997), and angiotensin II levels increase (Pedersen et al., 1985), which in theory could all cause an increase in $\dot{V}_E$.

The pregnant group has significantly lower values for [Na^+] and [K^+] at rest, for [Na^+] and [K^+] at 70% T_{VENT} and for [Na^+] at 110% T_{VENT}. These reductions are probably the result of the hemodilution that occurs in pregnancy. Blood volume in pregnancy increases by 40-50% over nonpregnant levels (Lund and Donovan, 1967; Pivarnik et al., 1990). The expansion of blood volume is due to an estrogen-mediated stimulation of the renin-angiotensin system, which in turn augments aldosterone secretion, and both Na^+ and water retention (Longo, 1983). Reduced [Na^+] and [K^+] values at rest in pregnancy have been observed previously (Kydd, 1931; Brandstetter and Scheuller, 1959; Lucius et al., 1970; Kemp et al., 1997). As expected, pregnancy did not cause reductions in [Cl^-]. This is also consistent with previous reports (Kydd, 1931; Machida, 1986; Kemp et al., 1997). The pregnant group exhibits a higher [Ca^{2+}] at rest, the 70% T_{VENT} work rate and the 110% T_{VENT} work rate, although this only reached significance at 70% T_{VENT} and 110% T_{VENT}. Then higher [Ca^{2+}] in the pregnant group is the result of the lower [Alb] resulting in fewer calcium ions being bound to albumin and thus more present in the free form (i.e., [Ca^{2+}]).

Although between group differences did not reach statistical significance, lower values for [La^-] for exercising pregnant women have been reported by others in association with work rates above T_{VENT} (Clapp et al., 1987; McMurray et al., 1988; Wolfe et al., 1994b). This has been attributed to several factors including reduced lactate production, dilution of lactate in an expanded blood volume and fetal lactate utilization as a metabolic fuel.

The lower [SID] in the pregnant group compared to the nonpregnant group at rest, as observed previously (Kemp et al., 1997), was a result of the lower values for the cations ([Na^+], [K^+]) in the pregnant group, while there was no difference in the anions between groups. The decrease in [SID] observed in both groups during the transition from rest to exercise at 110% T_{VENT} was primarily the result of an increase in [La^-].

The present results for [TP], [ALB], [GLOB] and A/G ratio also agree with previous findings. The [TP] decreases in pregnancy with the [ALB] decreasing while [GLOB] slightly increases (Eastman, 1930; Kemp et al., 1997; Kydd, 1931; Plass and Matthew, 1926). The overall result is an increase in the A/G ratio (Eastman, 1930; Kemp et al., 1997; Pivarnik et al., 1990).

54

The [H$^+$] calculated using Stewart's model was significantly different from the measured [H$^+$] in the pregnant group but not in the control group. The discrepancy between the calculated and measured [H$^+$] in the pregnant group may be the result of a change in the dissociation equilibria used in the Stewart equation. Dissociation equilibria can be altered by osmolality and strong ions, both of which are altered in pregnancy.

The measured [H$^+$] and the [H$^+$] calculated using the Stewart model were significantly correlated at all measurement times, except for the resting values in the control group. The low correlation in the control group at rest may be the result of a lack of variability within the control group at rest (range = 4.06 nEq/L).

In accordance with Stewart's physicochemical approach to acid-base balance any change in [H$^+$] must result from changes in one or more of the independent variables: PCO$_2$, [SID] or total weak acid. In our study the transition from rest to cycling at the 70% T$_{VENT}$ work rate causes a significant increase in the [H$^+$] concentration from 37.1 ± 0.3 nEq/L to 38.6 ± 0.5 and from 40.1 ± 0.5 nEq/L to 41.9 ± 0.7 in the pregnant and control group respectively. Our results suggest that the significant increase in [A$_{TOT}$] played a major role in the increase in [H$^+$]. However, the nonsignificant rise in PCO$_2$ in the pregnant group and the nonsignificant decrease in [SID] in the control group also played a major role in the rise in [H$^+$]. A nonsignificant rise in PCO$_2$ in the control group had a small contribution to the rise in [H$^+$]. In the pregnant group the [SID] actually increased attenuating the rise in [H$^+$]. The decrease in [SID] in the control group was the result of a rise in [La$^-$] and [Cl$^-$] that was greater than the rise in the cations due to exercise-induced hemoconcentration. In the pregnant group [SID] increased due to a smaller change in [La$^-$]. [A$_{TOT}$] also increased due to exercise-induced hemoconcentration. These findings are similar to those of Kowalchuk *et al.* (1994) who observed that while [H$^+$] increased at a work rate below T$_{VENT}$ the [SID] did not decrease and PCO$_2$ increased only transiently, then returned to baseline at 6 min. They did not measure [A$_{TOT}$], but concluded that a factor other than [SID] or PCO$_2$ caused the increase in [H$^+$] which means it can only be [A$_{TOT}$]. It appears that changes in [H$^+$] below T$_{VENT}$ are the result of a significant increase in [A$_{TOT}$], while nonsignificant changes in [SID] or PCO$_2$ exert a similar effect on [H$^+$].

The significant increase in [H$^+$] at the 110% T$_{VENT}$ work rate to 42.5 ± 0.8 in the pregnant group and 44.6 ± 1.0 in the control group were the result of a significant increase in [A$_{TOT}$] and significant decrease in [SID] in both groups. [A$_{TOT}$] increased as a result of exercise-induced hemoconcentration. The decrease in [SID] was the result of a significant increase in [La$^-$]. PCO$_2$ values at the 110% T$_{VENT}$ work rate did not differ significantly from those at rest and had the smallest influence on [H$^+$]. The control group exhibited a nonsignificant decrease in PCO$_2$ at the 110% T$_{VENT}$ work rate indicating respiratory compensation to the rising [H$^+$] at this work rate. These results also agree with those of Kowalchuk *et al.* (1994) who also found that PCO$_2$ did not change after 6 minutes of exercise at 120% T$_{VENT}$ in young men, while [SID] decreased at that level. Kemp *et al.* (1997) also found that a decrease in [SID] accounted for most of the increase in [H$^+$] in response to a maximal exercise test with changes in [A$_{TOT}$] also having a large influence on [H$^+$] and changes in PCO$_2$ playing a minor role. However, other studies have reported that exercise-induced increases in PCO$_2$ can have a major effect on [H$^+$] (Kowalchuk *et al.*, 1988a; Pieschl *et al.*, 1992; Weinstein *et al.*, 1992).

CONCLUSIONS

Our results indicate that values for arterialized plasma $[H^+]$ are lower in pregnancy compared to the nonpregnant state, at rest and during exercise above and below the ventilatory threshold. This is due to a lower PCO_2, due to an increased minute ventilation in pregnancy, and a dilution of the weak acid component due to an expanded blood volume in pregnancy. The mechanisms, however, for changes in $[H^+]$ in response to exercise remain unaltered by pregnancy.

The contributions of the independent variables to changes in $[H^+]$ differ above and below T_{VENT}. Below T_{VENT} (i.e., during steady-state exercise) the increase in $[H^+]$ is the result of a significant increase in the concentration of the weak acid component due to exercise-induced hemoconcentration. The increase in $[H^+]$ above the ventilatory threshold (i.e., during nonsteady-state exercise) is due again to an increased concentration of the weak acid component, as well as a decrease in the $[SID]$ due to rising $[La^-]$ from the increased energy metabolism. PCO_2 is held constant above and below the ventilatory threshold, although signs of respiratory compensation at the 110% T_{VENT} work rate were observed in the control group.

The measured $[H^+]$ was in close agreement with the calculated $[H^+]$, from the Stewart equation, in the control group but not in the pregnant group. The poor agreement between the measured and calculated $[H^+]$ in the pregnant group may be the result of altered dissociation constants in the pregnant group due to changes in osmolality and the strong ions. The dissociation constants used are best estimates from the literature and may not accurately reflect the true dissociation constants of the substantially altered pregnant state.

Our results show that exercise intensities in pregnant women which are comparable to those in nonpregnant women, produce lower $[H^+]$ in the pregnant women compared to the nonpregnant women. Thus, acid-base regulation during exercise is not compromised by pregnancy. Exercise in pregnancy does not produce abnormally high levels of $[H^+]$ in the maternal circulation that could have a potentially detrimental effect on the fetus. A study by Blechner *et al.* (1967) demonstrated that imposed increases in maternal $[H^+]$ up to a level that basically eliminated the normal maternal-fetal $[H^+]$ gradient caused no changes in fetal $[H^+]$. The values of $[H^+]$ produced in the maternal circulation as a result of the work rates above and below T_{VENT} in this study did not increase to a greater extent than the $[H^+]$ in the Blechner *et al.* study that eliminated the maternal-fetal $[H^+]$ gradient. This suggests that the increases in the maternal $[H^+]$ with the exercise intensities above and below T_{VENT} should not affect the fetal $[H^+]$. Thus, the results of this study support the hypothesis that exercise at intensities up to 110% T_{VENT} in pregnant women does not have a detrimental effect on fetal acid-base balance.

Note: *The original proposal for this study called for us to test 15 pregnant subjects and 15 nonpregnant control subjects. However, data are currently available from only 9 nonpregnant control subjects because of difficulties in obtaining arterialized blood samples because of their small veins (pregnancy causes enlargement of veins and we had few problems in this group). To solve this problem, we have constructed a special plexiglas chamber which allows us to heat the subject's hand using a hair dryer to promote vasodilation (specifications were provided by Dr. Ira Jacobs, Defence and Civil Institute for Environmental Medicine, Downsview, Ontario). This system is quite effective. Even though the existing sample sizes are sufficient to achieve our statistical gains, we will test an additional group of 6 nonpregnant subjects.*

Our proposal specified that we would collect blood samples for analysis of angiotensin II and arginine vasopressin levels. This has been done and a major batch of samples is currently being analyzed (results will appear in our final report.

FACTORS THAT HAVE HELPED/ HINDERED PROGRESS

Overall progress for this contract is progressing in close accordance with the original Statement of Work (Appendix A):

♦ Study #3 (Acid Base Physiology) is essentially finished.

♦ Study #1 (Cardiac Autonomic Function) is progressing well; the high performance liquid chromatography system is in good condition and the first batch of catecholamine samples will be analyzed in the near future.

♦ Methods for Study #2 (Respiratory Gas Exchange) have been finalized and local research ethics board approval has been received (Appendix B). A complete Safety Program Plan and human use materials is currently in an advanced stage of preparation and will be submitted very soon.

All three projects are in very good technical condition. The only important technical problem (cited above) that we have encountered is in consistently obtaining arterialized blood samples from nonpregnant control subjects. This problem has been solved by using a specialized chamber to warm the subject's hand to promote arterialization.

The role of recruitment of physically active pregnant subjects is the only other limiting factor that could affect progress. Typically, the role of recruitment increases as local physicians, people from the local sports/fitness community and the general public become more aware of the existence of the project. Thus, we feel that good progress has been made to date.

PUBLICATIONS TO DATE

M.Sc. Theses and Ph.D. Dissertations

Heenan, A.P. (Ph.D. Dissertation in Progress). The Exercise/Pregnancy Model: Applications to Respiratory and Acid-Base Physiology.

Avery, N. (M.Sc. Thesis in Progress). Effects of Human Pregnancy on Cardiac Autonomic Function at Rest and During Exercise Above and Below the Ventilatory Anaerobic Threshold.

Heenan, A.P. (M.Sc. Thesis, 1997). Acid-Base Regulation Above and Below the Ventilatory Anaerobic Threshold in Late Gestation.

Review Article

Wolfe, L.A., J.G. Kemp, A.P. Heenan and P.J. Ohtake. The exercise/pregnancy model: Applications to respiratory and acid-base physiology. *Can. J. Physiol. Pharm.* (in review).

Journal Publication

Amara, C.E. and L.A. Wolfe. Reliability of noninvasive methods to measure cardiac autonomic function. *Can. J. Appl. Physiol.* (Accepted pending revision, February 11, 1997.)

Abstracts

Heenan, A.P. and L.A. Wolfe. The exercise/pregnancy model: Comparison of physicochemical approaches to acid-base analyses. *Med. Sci. Sports Exerc.* 30:(in review), 1998.

Preston, R., A.P. Heenan and L.A. Wolfe. Physicochemical analysis of exercise-induced changes in [H$^+$] in human pregnancy. *Med. Sci. Sports Exerc.* 30:(in review), 1998.

Heenan, A.P. and L.A. Wolfe. Acid-base regulation above and below the ventilatory anaerobic threshold in late gestation. *Can. J. Appl. Physiol.* 22:26P, 1997.

Amara, C.E. and L.A. Wolfe. Effects of human pregnancy on cardiac autonomic activity above and below the ventilatory anaerobic threshold. *Med. Sci. Sports Exerc.* 29:S4, 1997.

III.   CONCLUSIONS

Work on this contract is progressing well and results from the first year of study suggest that all of our original technical objectives will be achieved. Findings to date from Study #1 indicate that higher resting heart rates in pregnant women result from lower levels of parasympathetic/vagal activity and perhaps a higher level of cardiac sympathetic activity. Evidence also exists to support the idea that heart rate responses to strenuous exercise above the ventilatory anaerobic threshold are blunted due to a lower level of cardiac sympathetic activity. If this is confirmed by additional study a scientific basis will exist for altering recommended pulse rate target zones for aerobic exercise in pregnancy. Specifically, it appears that the recommended target range should be narrower (eg., 15 *vs.* 20 beats/min) and the upper limit of the range should be reduced.

Results of Study #3 confirm that maternal plasma $[H^+]$ during strenuous exercise above the ventilatory anaerobic threshold is maintained at or below levels observed in the nonpregnant state. This may be due to less lactate production or dilution of lactate in an expanded maternal blood volume. there appears to be less need to offset the reduction in [SID] caused by blood lactate accumulation by increasing pulmonary ventilation in order to reduce PCO_2. From a practical viewpoint, it appears that healthy active pregnant women can perform short bouts of strenuous exercise above the ventilatory anaerobic threshold without causing excessive changes in $[H^+]$.

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V. APPENDICES

APPENDIX A

ORIGINAL
STATEMENT OF WORK

STATEMENT OF WORK

As a direct result of 10 years of experience in this area of study, the measurement systems, subject recruitment methods and personnel arrangements (nurses, technicians) described in this proposal are already in place. Current studies funded by Health Canada (N.H.R.D.P.) and the Canadian Fitness and Lifestyle Research Institute end on August 31, 1996, leaving us the month of September 1996 to prepare to begin data collection on October 1, 1996. (Subject recruitment efforts will not be interrupted.)

Another important aspect of the present studies is that the rate of data collection depends on the rate of subject recruitment. Pregnant subjects will enter the study on a staggered time basis as they approach the 34th week of gestation. In this context, the following are important target dates for initiation/completion of important tasks.

Month 1	Initiate data collection for Study #1 (Cardiac Autonomic Function) and Study #3 (Acid-base Physiology).
Months 1-6	Set up and test high performance liquid chromatography system for analysis of plasma catecholamines (epinephrine and norepinephrine).
Months 1-10	Complete data collection for Study #3 (Acid-base Physiology).
Months 1-10	Finalize computer programs (linear interpolation, ensemble averaging, mathematical modelling for Phase I, II and III oxygen uptake kinetics for Study #2 (Respiratory Gas Exchange Kinetics). Our computer programmer (W. Pearce) and colleagues from the University of Western Ontario (D.A. Cunningham, Ph.D. and J.M. Kowalchuk) with experience in measurement of $\dot{V}O_2$ are available to help us with this task.
Months 11-12	Perform statistical analysis for Study #3 (Acid-base Physiology).
Month 13	Initiate data collection for Study #2 (Respiratory Gas Exchange Kinetics).
Months 13-22	Complete data collection and biochemistry analyses for Study #1 (Cardiac Autonomic Function) and Study #2 (Respiratory Gas Exchange Kinetics).
Months 23-24	Perform statistical analyses for Study #1 (Cardiac Autonomic Function) and Study #2 (Respiratory Gas Exchange).

APPENDIX B

LOCAL HUMAN RESEARCH
ETHICS BOARD

APPROVAL FOR STUDY #2

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD REVIEW APPROVAL

Queen's University, in accordance with the "Guidelines on Research Involving Human Subjects. 1987," prepared by the Medical Research Council, requires that research projects involving human subjects be reviewed annually to determine their acceptability on ethical grounds.

A Research Ethics Board composed of:

Dr. A.F. Clark	Associate Dean, Medical Research Services Faculty of Medicine, Queen's University Director of Research, Kingston General Hospital (Chair)
Dr. B. Appleby	Community Member
Dr. L.E. Dagnone	Professor, Department of Emergency Medicine, Queen's University
Dr. N.J. Delva	Associate Professor, Department of Psychiatry, Queen's University
Dr. S. Irving	Psychologist, St. Mary's of the Lake Hospital
Dr. K. James	Associate Director, National Cancer Institute of Canada Clinical Trials, Queen's University Associate Professor, Community Health & Epidemiology
Professor E. Kauffman	Assistant Professor, School of Nursing, Queen's University
Dr. J. Low	Professor, Department of Obstetrics and Gynaecology, Queen's University and Kingston General Hospital
Dr. J. Parlow	Assistant Professor, Department of Anaesthesia Assistant Professor, Department of Pharmacology & Toxicology, Queen's University
Professor P. Peppin	Associate Professor, Faculty of Law, Queen's University Associate Professor, Department of Family Medicine, Queen's University
Dr. W. Racz	Professor, Department of Pharmacology & Toxicology, Queen's University
Dr. M. Schumaker	Professor, Department of Religious Studies, Queen's University
Dr. S.J. Taylor	Bioethicist, Faculty of Medicine, Queen's University and Kingston General Hospital; Assistant Professor, Department of Family Medicine, Queen's University
Dr. G. Torrible	Community Member

has examined the protocol and consent form for the project entitled **"Effects of Human Pregnancy on Respiratory Gas Exchange Kinetics Above and Below the Ventilatory Anaerobic Threshold"** as proposed by Dr. L. Wolfe of the School of Physical and Health Education at Queen's University and considers it to be ethically acceptable. This approval is valid for one year. If there are any amendments or changes to the protocol affecting the subjects in this study, it is the responsibility of the principal investigator to notify the Research Ethics Board. Any adverse events must be reported to the Chair within 48 hours.

Albert J. Clark
Chair, Research Ethics Board

Oct 1/97
Date